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SYNTHETIC PENICILLINS

PENICILLIN is the most widely used antibiotic. With increased medical facilities in villages and small towns, the demand for this antibiotic is likely to increase several fold in future. In western countries where there has been extensive, often indiscriminate use of penicillin, the increasing populations of resistant staphylococci is causing anxiety. When most of the laboratories were looking for new antibiotics that would be effective against resistant staphylococci, the research workers in the Beecham Laboratories, England, in collaboration with Prof. E. B. Chain, struck new paths of investigation on penicillin. In 1959, Batchelor and co-workers reported the isolation of the penicillin nucleus 6-amino-penicillanic acid (6-APA) from penicillin fermentations without the side chain precursors. This permitted the synthesis of a wide range of penicillins by the introduction of different side chains. It also gave the hope that it would be possible to synthesise penicillins similar to the cephalosporins active against Gram negative bacteria and other human pathogens against which the available forms of penicillin have no activity. Results of investigations in the last two years reported in literature indicate that this objective is yet to be achieved. None of the synthetic penicillins so far tested have proved to be clinically superior to penicillin G and penicillin V, which are produced by the mould by direct fermentation.

One of the anticipated results of the isolation of 6-APA was the synthesis of new penicillins that would resist penicillin degradation by penicillinase. The first semi-synthetic penicillin to be prepared on commercial scale from 6-APA was the mixture of D and L isomers of 6-(alpha-phenooxypropionamido) penicillanate also known as phenoxyethyl penicillin (BRL-152). This is sold under various trade names such as Broxil (Beecham) Syncillin (Bristol), Maxipen (Chas. Pjizer). Darcil (Wyeth), Chemipen (Squibb) etc. All are included under the generic name phenethicillin. In 1960, Beecham Research Laboratories announced another synthetic penicillin BRL-1241 or Celbenin, with the chemical structure sodium-6-(2, 6- dimethoxybenzamido) penicillanate monohydrate. This has been given the B. P. approved name sodium methicillin.

Since 6-APA was the starting material for synthesising new penicillin compounds, numerous investigations were started in different laboratories for obtaining high yields of the penicillin nucleus. 6-APA is obtained either by direct fermentation using *Penicillium chrysogenum* strains or by splitting penicillin G salts with enzymes produced by micro-organism. Much of the valuable scientific data on the production aspects are commercial secrets.

When potassium phenethicillin (Broxil) was introduced for clinical evaluation, it was found to be well absorbed when given orally, and produce penicillin blood levels atleast twice as high as that with penicillin V, and almost the same as that obtained by intramuscular injection of penicillin G. Recent work by Griffith and collaborators has shown that from the point of the amount of antibiotic in the blood, phenethicillin gives higher concentration than penicillin V, but when the antimicrobial activity for that amount of antibiotic present in the blood is assessed, phenethicillin is not superior to penicillin V. Further, phenethicillin is inactivated by penicillinase and is, therefore, of little value for treatment against resistant staphylococci.

The discovery of Celbenin or sodium methicillin has been acclaimed by most of the investigators as of considerable importance in strengthening our armory against resistant staphylococci. The antibiotic is not affected by staphylococcal penicillinase though slowly inactivated by penicillinase produced by *Bacillus licheniformis* and *B. cereus*.

Celbenin has, however, its own disadvantages—it has only one-fiftieth or one-hundredth of the activity of penicillin G against sensitive organisms; it has no value by the oral route and has to be given parenterally. The rate of excretion of Celbenin is quite fast, so that it has to be administered every 4 to 6 hours. Three cases of resistant staphylococci occurring naturally have been reported by Jevons from the Staphylococcal Reference Laboratory, London. These belonged to the group-III phage pattern, and all the three cases were found in the same hospital ward. It has been strongly recommended that sodium methicillin should not be used indiscriminately and must be reserved for emergency cases involving penicillin resistant staphylococci. As one investigator has pointed out, indiscriminate use has been avoided because of the high cost of the drug and its unsuitability for oral administration. The discovery of Celbenin has, however, given a lead for more potent synthetic penicillins in future.

REVIEW

Electrometric pH Measurement by Electronic Circuitry

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Introduction

CHEMICAL laboratory work and almost all industries require accurate pH measurements. The importance of pH measurement originated from the recognition of the role played by small variations of pH in chemical reactions and especially in biochemical processes. About four decades ago, when electronics was in its infancy, pH values of test solutions were determined by visually or optically observing the colour of an indicator in the unknown solution and comparing it with standard colours. Among other methods used may be mentioned the conductivity method, the electromotive force method, the freezing point method and the method of catalysis of ester hydrolysis. Since the rapid development of electronics and especially the technology of vacuum tube manufacture great strides have been made in the development of ultrasensitive instruments for the measurement of pH values.

Investigations of the ionic equilibria of water gave the concept of pH. A broad definition of an acid as an electron pair acceptor and of a base as an electron pair donor was enunciated by G. N. Lewis in 1938. A more practical definition, independently developed by Bronstead and Lowry in 1923, is that acids are substances having tendency to lose protons and bases those with properties of combining with protons to form acid. Thus an acid forms a conjugated system with a base represented by the reaction

$$\text{Acid} \rightleftharpoons \text{Base} + \text{proton}$$

$$HCl \rightleftharpoons Cl^- + H^+$$

Conjugation of an acid or base can generally be measured when the solvent combines with protons or supplies protons to combine with base. When the solvent has no acidic or basic properties the dissolved acids or bases will be present completely in a non-ionized state, Water, the commonest solvent, is amphoteric. In any dilute aqueous solution the concentration of non-ionized water is very large and is substantially constant. The ion constant product at 25°C derived from mass action expression of water equilibrium is 1.008×10^{-14}. In pure water the hydrogen ion and hydroxyl ion concentrations are equal and each of the value 1×10^{-7} molar at 25°C. For purpose of brevity and convenience as well as for theoretical reasons Sorensen (1909) defined $pH = -\log C_{H^+}$, where C_H represents the molar concentration of hydrogen ions. In the place of C_{H^+} we may write A_{H^+}, the activity of hydrogen ion. From these discussions it follows that solution of an acid will have a pH less than 7 and that of a base greater than 7. pH is then the actual acidity and alkalinity.

An important aspect to be taken into account in pH measurements is the buffering action of buffer solutions which are extensively used for standardizing and checking the calibrations of pH meters. Buffer action may be defined as the resistance exhibited by a solution to change in pH on addition of acid or alkali or upon dilution with more solvent.

Measurement of pH

To determine the hydrogen ion activity in a solution there are two methods: (1)

Electrometric, and (2) colorimetric. The first method depends on the measurement of the electromotive force of the galvanic cell, and the second method utilizes the property exhibited by acid base indicators of indicating different colours at different pH values. The amount of actual ionized hydrogen ions from acids of hydroxyl ions from bases being seldom equal to the titrable amount present in the solutions, the electrometric measurement of pH has advantages over the conventional titrimetric determinations. Further, in actual pH determinations, electrometric measurement saves time and labour. In the present paper the determination of pH using electronic circuitry is discussed.

The electrometric determination of pH depends on the measurement of the potential produced when an indicator electrode is dipping into the test solution. But, to be practicable, such a measurement has to be made with reference to a known value or a reference point that is taken as the datum line. Thus, the measurement of the potential of the indicator electrode is made relative to a reference electrode of known potential by means of a potentiometric circuit such that appreciable amount of current is not drawn from the reference cell nor polarization effect is produced at the surface of the indicator electrode. The pH meter is, therefore, simply a voltmeter modified so as to meet the stringent requirements necessitated by the special properties of the electrodes used for the measurements.

The characteristics, design features, etc., of the equipment used for the measurement of the potential developed by the electrode system depend largely on the nature of the electrodes. There have been in use a number of measuring or indicator electrodes such as the hydrogen, quinhydrone, antimony, and glass electrode. Of these the most widely used is undoubtedly the glass electrode.

A thin glass membrane of certain compositon* and containing some water will serve well as a conducting medium because of the presence of hydrogen (and deuterium) ions which move in and out of the surface of the glass. The glass membrane, in short, acts like a semi-permeable membrane to permit the movement of hydrogen (and deuterium) ions only depending on the composition of glass. This electrode resembles a concentration cell since any two solutions having a difference in hydrogen ion concentration and separated by a glass membrane, when there is a potential difference between them will tend to establish an equilibrium by electrolytic conduction through the glass. The potential difference between the two sides of the membrane is measured on a voltage measuring device, such as a potentiometer, by balancing the potential of the calomel reference electrode which dips into the test solution against that of a silver-silver chloride or calomel reference electrode dipping into the buffer solution inside the glass (membrane) electrode. The glass electrode consists of a small bulb of hydrogen ion sensitive glass approx. 0.2 mm. thick sealed to a stem made of a glass exhibiting very much greater resistance to transfer of ions other than H_2 (and D_2). Thus the ion transfer is confined to the glass membrane portion of the electrode and therefore the depth of immersion does not affect the measurements. The reference electrode inside the buffer solution is hermetically sealed within the glass bulb.

The resistance of glass electrode varies appreciably with temperature. The high resistance of the glass electrode ranging from 100 to 1,000 mega ohms at 25°C necessitates the connecting leads employed to have high insulation resistance and

*Glass used for manufacture of industrial electrode systems of good stability and low sodium error are reported to be of the following composition (in mole percentage): Silica, 63-67 ; Calcium and Barium oxides, 1-4 ; Lanthanum oxide, 2-3 ; Lithium oxide, 24-26 ; and Cesium oxide, 2.

good electrostatic shielding to eliminate transient electrical fields, leakage and capacity effects.

The hydrogen gas electrode is the standard reference electrode, but it is inconvenient to use and hence a calomel half cell is more widely adopted.

The potential of calomel electrode which normally contains potassium chloride solution depends on the concentration of KCl in the cell. To prevent contamination from the test solution the reference electrode is connected to the test solution through a salt bridge. A saturated KCl salt bridge is more often used because the liquid junction potential is small due to almost equal mobilities of potassium chloride ions.

The pH meter

The glass electrode being a source of very high impedence voltage has a very high resistance to the flow of current. Even the small flow of current may enhance polarization effects leading to variations in the concentration of ions at the electrode surface resulting in changes in the electrode potential. These factors, which depend on the properties of the glass electrode, make it necessary to ensure that the voltage measuring device does not draw any appreciable amount of current from the electrode in the process of measurement. Some of the approaches that have been made to tackle this problem effectively such that not more than 10^{-12} amp. of current is drawn, are by employing (1) a potentiometric circuit with vacuum amplifiers, (2) the electrometer tube, (3) chopper bar amplifier, and (4) vibrating reed condenser.

A conventional diagram of a potentiometer circuit employed for pH measurement is shown in Fig. 1. The battery E sends a constant current through the slide wire S establishing a voltage difference across it. This voltage is more than the highest voltage (14 m.v.) the meter is

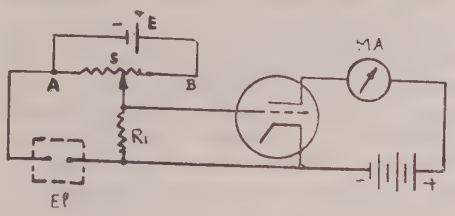

Fig. 1.
Potentiometric circuit.

expected to measure. The electrodes are connected at El and on moving the slide wire to the left or to the right the e.m.f. of the electrode system is opposed by the e.m.f. between A and S such that the grid of the tube is at the same potential as its cathode. This condition causes a certain amount of current to flow through a milliammeter (MA) connected in the plate circuit of the vacuum tube which is a standard deflection. During measurements the operator has only to adjust the slide wire such that the meter will read the standard deflection. The slide wire can be calibrated in terms of pH values, which is usually done on a circular scale. This method is generally known as the null deflection method. The same circuit can also be used to measure the pH directly on the meter. For this purpose, at zero input at El the potentiometer is adjusted and left as such. When the electrodes are connected a voltage will exist between the grid and cathode which will alter the current through the plate. The milliammeter can then be calibrated in terms of the pH values and read directly.

The above methods have their disadvantages. In the null balance method and in the direct reading method the current that flows through the resistance R_1 is sufficiently high to reduce the accuracy of pH determinations because of polarization effect and voltage drop in the resistance.

The effects of large current flow through the electrodes in potentiometric circuits may be overcome by employing electrometer

tubes in pH meters. Another advantage with electrometer tubes is that the potential difference of the electrodes could be measured directly without appreciable current drain. In principle the electrometer tube is essentially a vacuum tube with an extremely low grid current. The electron current flowing from cathode to plate in a vacuum tube is throttled by the grid voltage. If grid is more positive than cathode more current passes and vice versa. Hence, the electrode system of a pH measuring unit can be directly connected to grid and cathode and used for reading pH value directly. But in the ordinary vacuum tubes a small residual gas pressure remains (sometimes even after gettering). The gas when ionized due to electron bombardment may cause a grid current. In such tubes there are also leakage paths from grid to cathode across the envelop of the tube or the insulator carrying the socket pins. In order to minimize these adverse effects, in electrometer tubes high quality insulators* are used for the tube base, the pins are kept as far apart as possible from one another and the residual gas pressure is made extremely low. In addition, a positively charged screen grid is employed in front of the grid to shield it from positive ion bombardment. The cathodes of such tubes are made of special meterials such that very high electron emissivity is obtained even at low voltage between plate and cathode. Further, the grid is almost always worked at a positive potential such that the positive ions are repelled. This type of tubes can be used in either direct reading or null balance pH meters.

A brief discussion of the principle of DC amplifiers will help us understand why chopper bar and vibrating reed condenser amplifiers are used in the measurement of pH values. The two types of pH meters discussed above are purely DC amplifiers with the conventional resistance coupling only, before and between stages of an amplifier.

The principal difficulty in the design of DC amplifiers result from the inapplicability of capacitive or inductive coupling between stages. The problem of connecting the plate of one stage to the grid of the next directly is always serious because of chances of applying too high a potential on the grid in the absence of elaborate voltage divider arrangement. The more common method now adopted in such cases is the use of a bleeder with a number of taps and a regulated power supply. For each tube and each stage the correct potential is tapped and used.

Even with a well designed circuit and regulated power supply direct coupling between stages may result in excessive drift. The output meter will show a slow change even though the input is constant. This is caused by the gradual changes in supply voltage, capacitance and inductance. Cathode follower circuits and very high quality components of very low temperature coefficients, etc., have been used to reduce this drift.

A highly successful method of eliminating drift is by chopping or modulating the signal, which has the effect of converting the DC to an equal AC signal. The AC amplifier which is not subject to drift may then be used. In DC amplifier the plate current of a vacuum tube being dependent on the grid voltage as well as on the cathode to plate electron accelerating voltage, the plate current varies with plate voltage changes. However, if the signal to be amplified is an AC and the alternating component of the tube current is the part of the output of interest, then the amplification is much less dependent on plate voltage. Also, if the tube

*Thermoplastics and synthetic resins are widely used as high quality insulators because of their machinability and good insulating properties such as high dielectric strength, high volume and surface resistance as well as low power factor and generally low dielectric constant.

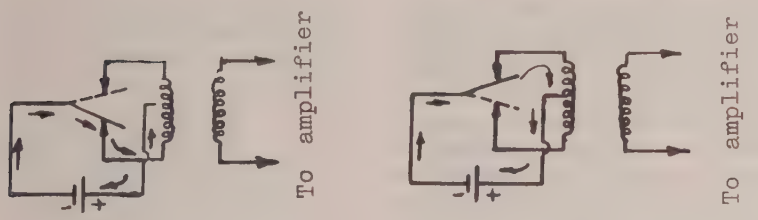

Fig. 2.
Conversion of DC to AC using chopper bar.

characteristic curve is approximately linear, the magnitude of oscillation of tube current will be approximately independent of the absolute magnitude of plate current and hence of plate voltage.

One of the methods of converting DC to AC is by employing a chopper bar as shown in Fig. 2. The source of voltage or the electrode system is connected to the centre tap of the primary of a transformer and the reed or chopper bar operated by an electromagnet. Depending on the frequency of operation of the electromagnet the chopper bar makes contact with the two contact points to which are connected the two terminals of the primary winding. As denoted by the arrows the current flows in the primary winding when the chopper bar makes contact with either point which is picked up by the secondary winding (magnified or unmagnified) and fed to the amplifier. After amplification the output is rectified and the DC signal applied to a meter and read as pH units. Some current, of course, usually flows in the chopper circuit when producing AC signal, but this can be minimized by keeping the impedence of the circuit sufficiently high.

Another method of conversion of DC signal obtained during pH measurements to AC is by the use of a vibrating reed condenser. This is also called a dynamic condenser and is shown in Fig. 3 and 4.

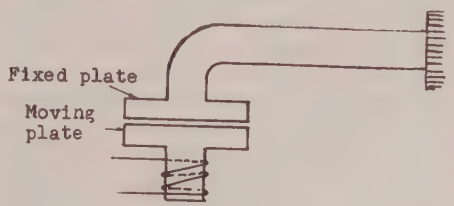

Fig. 3.
Conversion of DC to AC using vibrating reed condenser.

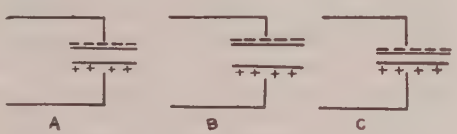

Fig. 4
Conversion of DC to AC using vibrating reed condenser.
A=Normal position of plates
B=Plates moved apart
C=Plates closed together

The system consists of a fixed plate and a moving plate which is vibrated by an AC energised electromagnet. The vibrations produce periodic variation in the condenser spacing and hence in the capacitance which results in a fluctuating voltage. The advantage of dynamic condenser over chopper bar is that in the former current flow is practically very low compared to the significant flow of current in a chopper bar.

The relationship between charge, capacitance and voltage across the condenser plates is given by the equation Q=CV, where Q is the charge, C the capacitance, and V the voltage between the plates of the condenser. The capacitance is a function of the shape, area and separation between the two plates. Hence, when one plate of the condenser is fixed and the other vibrated by means of an electromagnet, the capacitance fluctuates at the frequency of the vibration. This causes a fluctuating voltage which is applied to the grid and cathode of an RC coupled amplifier or a transformer coupled amplifier and the amplified output is measured in terms of pH. Usually the glass and reference electrodes are connected to the plates of the condenser and the same points connected to the amplifier thus maintaining a fluctuating input to the amplifier which is proportional to the DC potential due to the pH of the solution. It is commonly known that for high reproducibility of results and accuracy of any electronic measurement using amplifier and oscillators, the circuits should be of a very stable nature. Even the use of highly stable components and regulated power supply may not at times meet the demands of high stability especially after the circuit has been in use for long periods. As the components get aged the characteristics of these vary and naturally the stability departs from the normal. The adoption of negative feedback principle in circuits helps a great deal to offset the adverse effects of ageing of components and imparts high stability to the circuit. This further makes the amplification of the circuit practically independent of variation in the properties of the vacuum tubes. The fundamental principle involved in the method of negative feedback stabilization of an amplifier is shown in Fig. 5.

In Fig. 6 is shown the decreased amplification due to ageing of vacuum tube. The net output is a function of input, amplification factor and feedback. Any decrease in the amplification factor is compensated by a corresponding decrease in the magnitude of feedback voltage thus leaving the net output voltage constant. It can also be represented mathematically. If E_{in} is the input to amplifier whose output is E_{out}, the amplification factor be A, and the fraction of output fed back in opposition to input be n, then

$$A \times E_{in} = E_{out}$$

when there is no feedback; $E_{in} - nE_{out}$ is the new input when feedback is effected. The net output after amplification would be $AE_{in} - AnE_{out}$. Hence $E_{out} = AE_{in} - AnE_{out}$. Rearranging this we get,

$$\frac{E_{out}}{E_{in}} = \frac{A}{1 - An}$$

If the amplification factor is very large and feedback small,

$$\frac{E_{out}}{E_{in}} = \frac{1}{n} \quad \text{constant}$$

Thus the negative feedback acts automatically as a built-in correction factor adjust-

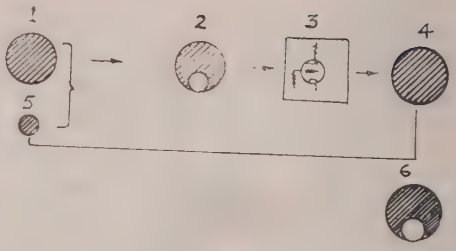

Fig. 5.
Negative feedback stabilization of amplifier

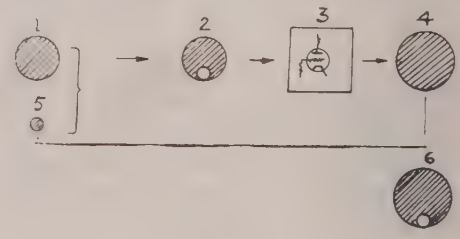

Fig. 6.
Decreased amplification due to ageing of vacuum tube

(1) Input voltage. (2) Net input. (3) Amplifier. (4) Total output. (5) Feedback. (6) Total output minus feedback.

ing for the variations in the amplifier characteristics. At the same time it maintains an output which is a faithful reproduction of the input signal amplified to a constant level.

Commercially available pH meters make use of one of the four principles of measurement discussed above. Between manufacturers design features of the equipment may vary. One manufacturer may use dry battery for the power supplies in connection with a potentiometric circuit while another manufacturer may make use of the same principle but with a regulated and rectified power supply derived from the mains. The disadvantage of dry cells is that their life is limited and the batteries have to be changed often. The supply from the mains, on the other hand, has unlimited life, but fluctuations due to variations in the mains voltage (though the supply might have been regulated) and errors due to ripples present in the rectified power, etc., may result. The adoption of chopper bar amplifier or vibrating reed condenser in pH instruments, which are recent developments apart from transistorized circuits, have advantages over other types. However, it should be borne in mind that most accurate measurements are obtained only by the use of potentiometric circuit employing null balance method. This is so because at the null balance position the current drawn is practically nil and hence no voltage drop or any polarization occurs. But, as already mentioned, this is a tedious method. The accuracy of the null balance method lies in the capacity of the potentiometer to resolve the voltage to very minute quantities of the order of 0.02 to 0.05 m.v. per division, whereas the accuracy of the direct reading meter lies in the accuracy with which voltage can be read on the indicating meter.

Care and maintenance of glass electrodes

The general care and precautions necessary in any measurement are equally important in pH measurements. The buffer solution used for routine standardization of the meter should be of a high order of purity. The electrodes must be kept as clean as possible in distilled water when not in use. Before and after each measurement the electrodes should be washed carefully in distilled water and wiped off with absorbent tissue paper or cotton. The glass electrode in particular must be thoroughly washed as glass surfaces are not rapidly cleaned and they are excellent ionic sorption materials. It may be advantageous to keep the electrodes immersed in test solution for some time, and the solution stirred well before taking final readings because there is often a time lag before equilibrium conditions set in. Even a small crack in the glass electrode is sufficient to give erroneous readings and in such cases the only remedy is to replace the electrode. Old glass electrodes may be renovated in certain cases by dipping them for one minute in 20 per cent ammonium acid fluoride solution so that the electrode gets etched.

Maintenance of saturated KCl electrode is comparatively easier. Whenever necessary the KCl solution can be replaced with fresh saturated KCl solution with a few crystals of KCl in it.

Measurement and control of pH in industry

Some of the special problems relating to the measurement and control of pH in industry in general and in fermentation processes in particular, are discussed below.

In laboratory pH measurements a high order of accuracy between 0.01 and 0.005 of a pH unit is essential. In the industry, on the other hand, accuracy is not the only criterion. In continuous industrial process stress is also laid on the strength of the components of the measuring system, particularly of the electrodes so that breakdowns are at a minimum. In designing a pH control system for an industrial

process, the damage to the system that may result from the following factors have to be taken into account :

1) High operating temperature,
2) high pressure, and
3) corrosive nature of the fluids with which the system may come in contact.

Glass electrodes used for measurements at high operating temperatures should have (*a*) suitable glass composition for the bulb to prevent chemical attack, (*b*) high performance characteristics of the inner electrode, and (*c*) buffer solution of very stable nature. To reduce sodium ion errors, glass electrodes used for industrial purposes (operating at high temperature) should not preferably be of soda lime glass, because at high temperatures the water of the buffer solution evaporates and condenses on the inner stem of the electrode, and when the condensate flows back it leaches out the alkali of the glass thus leading to variation of the buffer value. Such variation can be reduced by using a buffer solution with non-varying ion concentration. It has been suggested that use of 0.2*M* buffer salt concentration instead of 0.05*M* also helps to check the variation in the buffer value. In addition, use of a glass electrode with the inner stem treated with silicone reduces the leaching effect to the very minimum.

At high temperatures the reference electrode exhibits a hysteresis effect. The calomel reference electrode undergoes many reactions at transient as well as steady temperatures. One method of eliminating such effects is by locating the reference electrode away from the hot measuring fluid and by providing a liquid junction at the end of a long tube containing saturated potassium chloride. The stability of reference electrode can be vastly improved by the use of emulsified mercury (in the form of finely divided particles of mercury) such that the mercuric chloride formed easily gets converted to mercurous chloride.

An industrial form of reference electrode (Fig. 7) consists of a short piece of 5 mm. bore lead glass tube closed at one end with a short piece of platinum wire formed into a helix inside the tube and sealed to

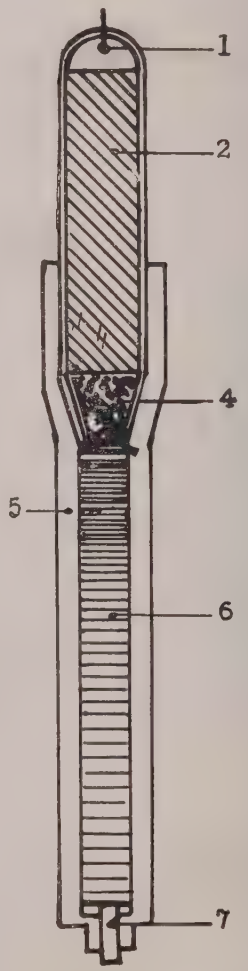

Fig. 7.
Industrial reference electrode
(1) Pt spiral with Hg globule.
(2) Hg_2Cl_2/Hg emulsion (electrolytic) with KCl solid.
(3) Glass tube.
(4) Cotton wool plug.
(5) Flexible rubber sac.
(6) KCl saturated soln. and KCl solid
(7) Porcelain rod

the closed end. The helix inside the electrode is thoroughly amalgamated and contains a small bead of mercury surrounding it. A paste of electrolytic mercury/calomel emulsion and finely crystallized KCl are added into the tube so that it is tightly packed. The mixture is retained in the tube by a cotton wool wad. A flexible long rubber tube is connected to this tube. The space inside this rubber tube is filled with saturated solution of KCl containing solid KCl. This forms the salt bridge. At the free end of the rubber tube is fitted a porcelain rod tightly enough to minimize the diffusion rate and maintain the liquid junction.

In measuring pH at high pressure conditions, the reference electrode is the one which is mainly affected. At high pressure the measured liquid may enter the reference electrode contaminating the saturated KCl. The resulting variation in the potential of the reference electrode may be avoided by the following methods. One method is to bleed off a small sample of the measured liquid at atmospheric pressure. But this cannot be done where the measured fluid is under sterile conditions as would be in most of the fermentation processes. Under such stringent conditions the usual practice is to have a remote reservoir of KCl solution located above the process liquid level. This reservoir supplies KCl to the reference electrode under a pressurised system (air under pressure) above the KCl column, or the reference electrode and the salt bridge can be completely filled with KCl solution and sealed in a flexible tubing which equalises the pressure inside and outside.

At times when the measured liquid is of low conductance the reference electrode develops high resistance. In such cases the reference and measuring electrodes can be connected through a bucket condenser which charges to the voltage between the electrodes. By employing a double pole switch arrangement the condenser is disconnected from the electrodes and connected to the electrometer circuit for measurements, at regular intervals. This prevents galvanic e.m.f. between earth and liquid which would otherwise occur if the electrode system is directly connected to the electrometer tube. In the latter arrangement the earth side of the electrometer will have to be connected through the earth to the reference electrode and measured solution.

The housing and casing for the electrode system should be of such type as to prevent water vapour entering it. Water vapour allowed into the casing may affect the electrical insulation. Further, the housing used should be capable of withstanding thermal and mechanical shocks as well as corrosion. Metal is one of the best materials for the casing to meet most of these requirements.

As an example from the industry the measurement and control of pH in penicillin manufacture is discussed below. During fermentations in the large tanks the pH value of the broth starts rising above the neutral value after about 50 hours and reaches a maximum of 8.4. To maintain the pH at neutral calcium carbonate is used as buffer in cases where no automatic pH control is employed. Manual control of pH by addition of acid into the tank is ruled out since after addition it is not advisable to draw out samples to check the pH value at frequent intervals. A number of precautionary measures have to be taken to prevent any possible contaminations from outside while taking samples and hence only at long intervals samples may be drawn. Automatic control of pH is, therefore, desirable and preferred.

A schematic diagram of the pH control system in fermentation is shown in Fig. 8. The broth from the bottom of the fermentor flows by gravity through a chamber (pH-T, pH transmitter) containing the electrode system and temperature compensator.

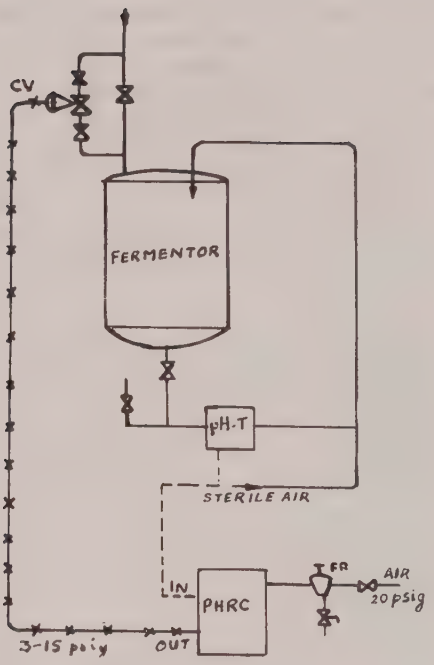

Fig. 8.
pH control system in fermentation

From here the broth is lifted up by means of a pump or compressed sterile air and poured back into the fermentor at the top. The electrodes or pH transmitter develops a potential proportional to the pH of the broth and transmits an electric signal (broken lines in the diagram) to the pH recorder controller (PHRC). Inside the recorder controller a baffle, operated by a moving coil to which is applied the signal from the pH transmitter, moves across a nozzle through which air bleeds. Depending on the position of the baffle and nozzle the air flow from the nozzle is controlled giving an output which varies from 3 and 15 psig. which is the back pressure in the tube containing the nozzle. This pneumatic signal is transmitted to the control valve CV. The controller may be one having a proportional plus automatic reset (integral) so that it will respond quickly to any load change. The control valve function chosen is such that in case of instrument-air-supply failure the valve will close completely preventing any "over addition" of acid. The controller function chosen is direct acting such that with a rise in pH more air is supplied to controller thus opening the valve further. This causes additional acid or neutralising fluid to flow into the fermentor maintaining the pH value at normal. With such an arrangement it has been claimed that, in practice, pH value could be controlled within a close limit of 0.1 pH unit.

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A Device For Continuous Feeding of Nutrients in Large Scale Fermentations

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IN many industrial fermentations it is necessary to add small quantities of materials to the batch in the form of liquid or solution during the fermentation. In antibiotic fermentations such additions must be made under absolutely sterile conditions in order to maintain the pure culture status. The material to be added may be batch sterilized, as is the common practice, and held under pressure in a storage vessel, or it may be continuously sterilized and cooled. In either case the feeding of sterilized extra medium to a pre-sterilized fermentor containing sterile air under moderate pressure presents difficulties. The additions may be made by any one of the following methods :

(*a*) Regulated additions through a calibrated vessel on a pre-arranged schedule. This method has the obvious disadvantage of being an intermittent operation. Attempts to make it a continuous operation have not been successful on account of the greater dependence on the human element.

(*b*) In continuous addition the major advantage would be elimination of the human factor to a considerable extent, and less variation in the nutrient concentration in the fermentation medium. A continuous or near-continuous addition may be effected with sterile feed pump. The standard diaphragm pumps have sufficiently low rates for satisfactory metering of sterile liquids. Peristaltic action pumps, which press the liquid through a sterile tubing, have also been used successfully in the continuous feeding of liquids in such cases where accurate control of flow rate is not important. A plunger type metering pump in which the stroke of the pump can be controlled by the operator, can also be used. A Vanton sealless gland pump with Flex-i-line of neoprene was tried for feeding one of the fermentors, but abandoned due to frequent mechanical trouble and damage to liner. In using these mechanical devices frequent check on the flow rate is difficult unless the liquid is channelled through a rotameter. Maintenance of sterility inside the rotameter and its packing is again a problem. Further, when large number of fermentors are to be fed the initial investment cost on sterile pumps would be high.

In our attempts to solve the problem, a simple device with no moving parts unlike in pumps, which would allow an intermittent addition, though not a continuous flow, of liquid at short intervals, was developed and successfully put into operation for feeding sugar solution to the fermentor. The equipment consists of a tantalus cup arrangement with a siphon tube inside, which allows a clear liquid to be fed into the fermentor every few minutes (Figs. 1 & 2). Neither the siphon nor the tantalus cup, it should be noted, control the flow. The tantalus cup facilitates checking of the flow rate at any particular instant, while the flow is controlled by a good needle valve of appropriate dimensions.

The main theoretical considerations in the choice of a siphon tube of suitable dimensions may be summarized as follows :

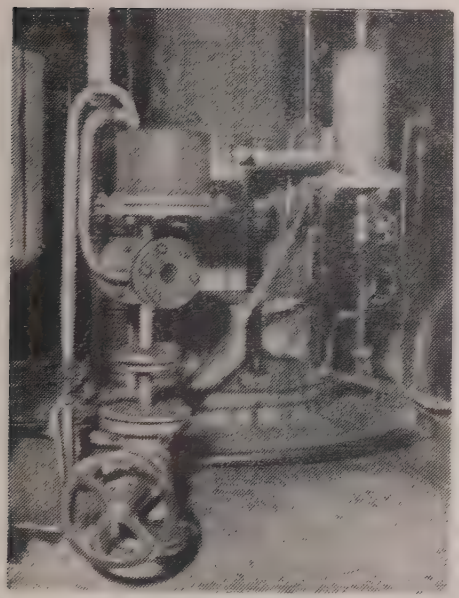

Fig. 1. "Tantalus cup" continuous feeder and strainer.

1. When the siphon tube is too large for a particular rate of flow of the liquid, it merely functions as an overflow pipe and the liquid discharges continuously when its level is at the top of the tube. An attempt was made to overcome this difficulty by providing a loop in the discharge leg of the siphon. It was, however, found that if there was overflow in the siphon proper, a similar overflow occurred from the loop also, and this could not be easily corrected. This sets a limit on the minimum flow rate for a siphon tube of given dimensions if it is to work as a siphon and not as an overflow pipe.

2. On the other hand, if the siphon tube is too small for the required rate of flow, the siphon may not empty out com pletely. A slight decrease in the flow rate may cause the discharge to break off, whereas an increase in its rate will result in the rise of the liquid inside the cup to set new equilibrium. In such cases the liquid level inside the tantalus cup will automatically adjust itself such that the inflow equals the outflow. This gives the maximum rate of flow below which the siphon will function satisfactorily.

3. Within the limits mentioned the flow of the liquid in the siphon may continue when the level is at the lowest inside the cup. This is possible due to (a) "gaede" effect when the discharge leg of the siphon is more than twice the length of the suction arm; and (b) various other factors mentioned later in this paper.

From the above considerations it follows that for a given size of the siphon tube there will be a maximum and a minimum rate of flow of liquid. The maximum and minimum flow rates depend on the viscosity and surface tension of the liquid handled. Higher the viscosity lower will be the maximum value of the flow rate as determined by (2) above, and lower the surface tension higher will be minimum value of the flow rate as deduced from (1). For a liquid with high viscosity and low surface tension, the difference between the maximum and minimum flow rates may be small or even negative. In such cases the siphon cannot work as a discontinuous one at any flow rates. Normally, liquids with high viscosity coefficient have a high surface tension also. Mixtures of certain compounds, however, have a high viscosity and low surface tension. Sugar solution with which the present experiments are mostly concerned, is an example. Addition of sugar to water lowers the surface tension to a small extent but raises the viscosity considerably. Another example of a liquid with high viscosity and low surface tension is glycerol. At 18°C, 100 per cent glycerol has a surface tension slightly lower than that of water but the viscosity is about 1,500 times higher. Such a liquid cannot be handled by the siphon arrangement.

In selecting a siphon tube of suitable dimensions our objective was not only to overcome the continuous flow at the top

and bottom levels but also to get the widest margin between the two limits within which the siphon will function satisfactorily for the desired flow rate of 28 litres per hour. Experiments carried out with siphon tubes of various dimensions are discussed below.

Two M. S. tubes of $\frac{3}{8}''$ and $\frac{1}{4}''$ nominal bore size were selected in the experiments for feeding water, and a mixture of sugar solution and amonia precursor (SAP) The length of the discharge leg was varied, but care was taken to avoid "gaede" effect. Investigations were also carried out with orfice plates of different sizes fitted at the discharge end. Results from the first set of experiements are presented in Tables I to IV.

From Table I it would be seen that reducing the discharge opening tends to reduce the margin between the minimum and maximum flow rates. Even within the limits of flow rate, a continuous flow was often noticed at the end of siphoning. In Table II the higher value for the minimum flow rate was due to the defective bend of the tube. It was not possible to get a sharp bend of uniform cross-section.

For the second set of experiments a siphon made of stainless steel tubing, 5/6″ internal diameter, and 1/2″ bend centre to centre, was chosen. The smaller bore size and sharper bend gave as expected, a lower value for the minimum flow rate. However, a continuous flow at the end of

Table I

Siphon Tube M. S., $\frac{3}{8}''$. Bend, $2\frac{1}{4}''$ Centre to Centre. Suction Arm, 4″. Discharge Leg, $2\frac{3}{4}''$

Discharge opening	Water		SAP	
	Minimum flow rate 1/hr.	Maximum flow rate 1/hr.	Minimum flow rate 1/hr.	Maximum flow rate 1/hr.
Original 3/8″	17.8	198.0	19.8	160.0
Orfice plate 5/16″	19.68	117.6	24.96	99.6
Orfice plate 1/4″	15.24	75.6	20.4	77.4

Table II

Siphon Tube M. S., 3/8″. Bend, 7/8″ Centre to Centre. Suction Arm, 4″. Discharge Leg, 4″

Water		SAP	
Minimum flow rate 1/hr.	Maximum flow rate 1/hr.	Minimum flow rate 1/hr.	Maximum flow rate 1/hr.
24.6	Continuous flow at end of siphoning	25.4	Continuous flow at end of siphoning

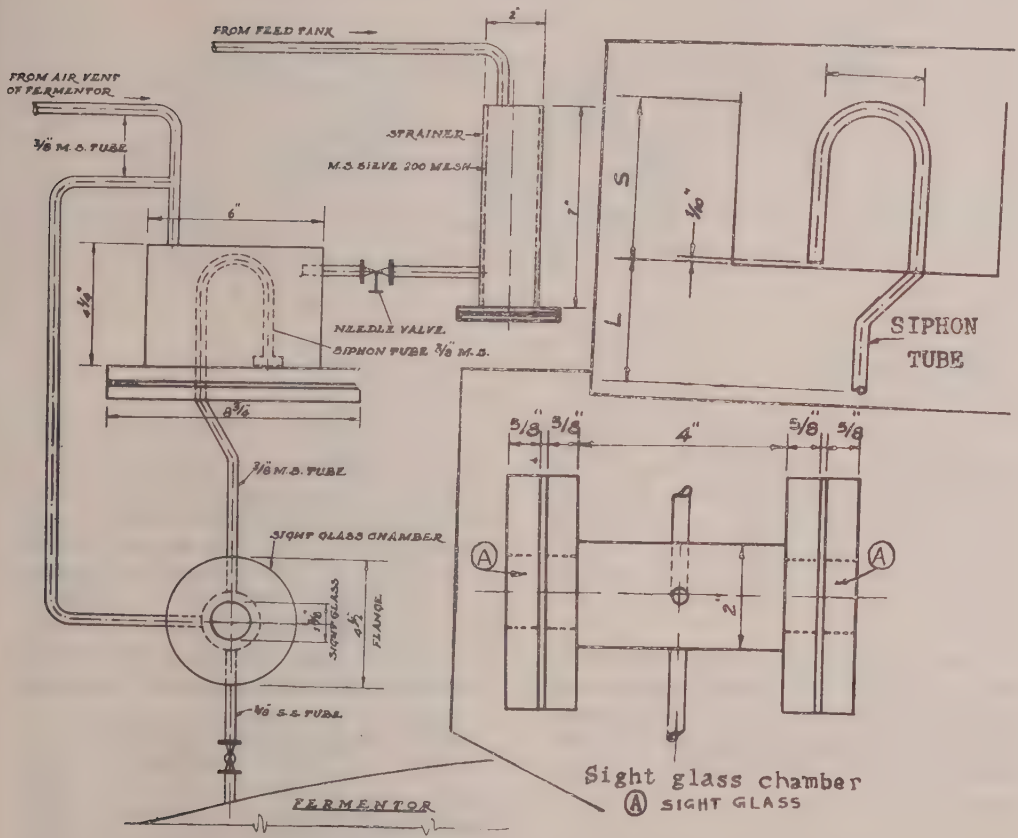

Fig. 2. Schematic diagram of "tantalus cup" feeder and strainer fitted to fermentor.
Top inset: Siphon tube. S=Suction arm; L=Discharge leg.
Lower inset : Sight-glass chamber.

TABLE III

SIPHON TUBE M. S., 1/4." BEND, 1½" CENTRE TO CENTRE. SUCTION ARM, 4." DISCHARGE LEG, 4"

Water		SAP	
Minimum flow rate 1/hr.	Maximum flow rate 1/hr.	Minimum flow rate 1/hr.	Maximum flow rate 1 hr.
21.10	Continuous flow at end of siphoning	25.0	Continuous flow at end of siphoning

TABLE IV

SIPHON TUBE M. S., 7/16″ NOMINAL BORE. BEND, 1½″ CENTRE TO CENTRE

	Water		SAP	
	Minimum flow rate 1/hr.	Maximum flow rate 1/hr.	Minimum flow rate 1/hr.	Maximum flow rate 1/hr.
Suction arm, 4″ Discharge leg, 4 3/16″	42	Continuous flow at end of siphoning	51	Continuous flow at end of siphoning
Suction arm, 4″ Discharge leg, 4″	36	-Do.-	37	-Do.-
Suction arm, 4″ Discharge leg, 4 3/16″ M. S. ring held in position by rubber	37	-Do.-	36.9	-Do.-

siphoning proved this tube to be of no practical use. Length of the discharge leg was varied but without any success in preventing the continuous flow. Results of the second set of experiments are presented in Tables V and VI.

A slanting cut at the suction end of the tube made no appreciable improvement except that the values for the minimum rate of flow were higher (Table VI). The latter rise in the values could not be attributed to any particular factor.

Roughening the inside surface of the tube by caramelizing sugar solution did not help in either lowering the value for the minimum rate of flow or in preventing continuous flow.

The continuous flow which invariably occurred in the above experiments could be explained as due to admission of insufficient air at the end of the discharge cycle. If only few air bubbles enter the siphon tube, the siphon continues to discharge liquid though at a reduced rate. The level of the liquid adjusts itself to an equilibrium when the outflow becomes equal to inflow.

Among the possibilities for admission of insufficient air may be insufficient turbulence at the end of the discharge cycle. If the liquid is let in so that it flows along the sides of the cup or down the siphon leg, the liquid level will be free from turbulence and only a limited quantity of air drawn in. It was also observed that when the inside surface of the tube was dry as

TABLE V

	Water		SAP	
	Minimum flow rate 1/hr.	Maximum flow rate 1/hr.	Minimum flow rate 1/hr.	Maximum flow rate 1/hr.
Suction arm, 4″. Discharge leg, 5 1/2″	9.1	Continuous flow at end of siphoning	11.6	Continuous flow at end of siphoning
Suction arm, 4″. Discharge leg, 3 9/10″	8.48	-Do-	15.6	-Do-
Suction arm, 4″. Discharge leg, 2 3/5″.	8.6	-Do-	12.36	-Do-

TABLE VI

	Water		SAP	
	Minimum flow rate l/hr.	Maximum flow rate l/hr.	Minimum flow rate l/hr.	Maximum flow rate l/hr.
Suction arm, 3 9/10″. Discharge leg, 4″	17.0	Continuous flow at end of siphoning	19.8	Continuous flow at end of siphoning
Suction arm, 3 7″/10. Discharge leg, 4⅛″.	13.8	-Do-	15.6	-Do-

TABLE VII

Siphon tube M. S., ⅜″ I. D., with	Water		SAP	
	Minimum flow rate l/hr.	Maximum flow rate l/hr.	Minimum flow rate l/hr.	Maximum flow rate l/hr.
Enlarged suction end, funnel type	15.0	120.0	15.6	100.0
Enlarged suction end, cup type	13.0	120.0	15.5	120.0

would be at the beginning of the experiment, the results were unpredictable.

The positive means tried to break the siphon discharge at the end of the cycle were :

(a) Constricting the disch arge end of the siphon with orifices, discs, wire, etc. None of these manipulations gave satisfactory results.

(b) Suction end of the tube was enlarged by welding an inverted funnel or an inverted cup. This gave best results. The enlarged suction end permitted a larger volume of air into the tube at the end of siphoning.

(c) The inlet line of the liquid was extended inside the cup by about 1/2″ to prevent the liquid running along the side. The turbulence created by the falling liquid inside the cup resulted in breaking the liquid adhering to the suction end thereby admitting larger volume of air into the tube. The experimental results (Table VII) obtained with the enlarged suction ends were satisfactory for feeding sugar solution and SAP into the fermentors.

The siphon tube as modified was fitted on to a fermentor (Fig. 2). After a few trial runs it was found that the needle valve, which is the controlling element, got frequently choked up with solid matter in the sugar solution. The solid material may come from sterilized sugar solution as there will be certain amount of caramelization during sterilization of the solution. It was also felt that on an industrial scale it would be difficult to avoid the presence of fine solids in the solution. An oversize strainer of 200 mesh M. S. wire cloth rolled up as a candle was introduced in the SAP line to the tantalus cup. The performance of the siphon tube was then trouble free and needed very little attention during a number of fermentation cycles. The titres obtained with continuous addition of nutrients are comparable with those obtained in batch additions. As has been mentioned, the major advantage of continuous addition is the elimination of dependence on human factor to a great extent, and lesser chances of variation in sugar concentration in the fermentor medium.

Hamycin, a new Antifungal Antiobiotic

I. DISCOVERY AND BIOLOGICAL STUDIES

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AN actinomycete isolated from soil samples collected in Pimpri, Poona, produces a new polyene type antibiotic[1,2] along with four other antimicrobial substances[3]. The species is aerobic, mesophilic, growing readily on most of the nutrient agar media employed. The submerged mycelium has various shades of yellow colour on different media used, and the aerial mycelium is greyish to pale buff coloured, bearing few imperfect spirals and spherical spores. The species is closely related to *Streptomyces celluloflavus* Nishim. and Kimura[4], but differs from it in several biochemical characters. Comparative studies indicate that the hamycin producing species represents an undescribed species, and the name *Streptomyces pimprina* Thirum. is proposed for it. A comparative account of the growth characteristics of *S. pimprina* and *S. celluloflavus* on different media is given in Table I and utilization of carbon compounds[5] in Table II.

TABLE I. CULTURAL CHARACTERS OF *S. PIMPRINA* AND *S. CELLULOFLAVUS*

Media	*S. pimprina*	*S. celluloflavus*
Synthetic agar (Czapek's)	Growth thin, transparent, submerged mycelium greenish-yellow, with greenish yellow soluble pigment.	Glossy growth developing into medium, later becoming marguerite yellow. Faint sulphur-yellow soluble pigment.
Glucose-agar	Growth moderate, flat submerged mycelium chesnut brown with pinkish tinge, aerial mycelium white at first, later pale greyish white with few imperfect spirals, soluble pigment pinkish.	Cream coloured to yellow growth, scant cottony white to grey aerial mycelium. Soluble sulphur yellow pigment.
Glucose asparagin agar	Growth moderate, flat, submerged mycelium pale yellow, no diffusible pigment, aerial mycelium greyish white.	——
Calcium malate agar	Growth thin and spreading with violet tinge, aerial mycelium scanty as white fluff, no diffusible pigment.	Primrose-yellow growth, later turning white to pale olive-buff, soluble citron-yellow pigment.
Starch plate	Growth moderate, spreading as deep violet crust, aerial mycelium absent, brownish-violet soluble pigment diffusing into medium, hydrolysis poor.	Smooth surface, abundant cream coloured growth. Aerial mycelium powdery white to olive-buff, soluble red yellow pigment, hydrolysis strong.

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TABLE I—(contd.)

Media	*S. pimprina*	*S. celluloflavus*
Tyrosine agar	Growth poor, as pale yellow crust, aerial mycelium scanty, no diffusible pigment.	Growth moist ivory-yellow to cream-buff, aerial mycelium scanty, soluble greenish-yellow pigment.
Nutrient agar	Growth poor, pale yellow, aerial mycelium absent, no soluble pigment.	Growth moist, olive-buff to colourless. Aerial mycelium scant white, yellow to old gold soluble pigment.
Gelatin	Growth poor, not liquified.	Liquifaction rapid to medium, growth ivory-yellow to olive-buff.
Litmus milk	Growth moderate, litmus slightly reduced and slightly coagulated.	Growth good, naphthalene yellow, reddish-brown soluble pigment, white aerial mycelium, peptonization begins after coagulation.
Potato plug	Growth profuse, folded whitish-yellow to greyish white. Aerial mycelium scanty, potato turning yellow.	Growth wrinkled, spreading, glistening, deep buff powdery-white aerial mycelium, potato turning deep olive-buff.
Carrot plug	Growth profuse, wrinkled, greyish-white, no colour for carrot.	Growth spreading, wrinkled olive-buff to dark buff, colour of plug unchanged.
Cellulose agar	Growth poor or absent.	Growth poor, developing into the medium, aerial mycelium none, soluble pigment yellow.
Emerson's medium	Growth fair, submerged mycelium cream-yellow with pinkish border, no soluble pigment, aerial mycelium abundant and white.	——
Reduction of nitrates	Poor, or not reduced to nitrite.	Reduced to nitrite.
Antagonistic properties	Produces the heptaene hamycin along with thiolutin, aureothricin, *iso*-butyro-pyrothine and a compound not belonging to thiolutin group.	Produces aureothricin.

Antibiotic production

Production of the antibiotic was first carried out in shake flasks and later in 500 gallon fermentors. The seed medium consisted of (in percentage) cornsteep liquor, 1; starch, 2.5; ammonium sulphate, 0.5; and calcium carbonate, 0.5; The pH was adjusted to 6.5 and the seed flasks were incubated at 28° on rotary shaker (250 r.p.m., 2″ throw). In the production medium 3 per cent glucose was used in the place of starch and 3 per cent peanut meal was used as nitrogen source. Yields of hamycin up to 350 μg/ml. were obtained.

In vitro **antimicrobial activity**

Pure hamycin was used for studying the antifungal spectra, and a large number of fungi including human and plant pathogens were tested. For determining the activity against filamentous fungi, the antibiotic was incorporated in agar media and the test

TABLE II. UTILIZATION OF CARBON COMPOUNDS

Carbon source	*S. pimprina*	*S. celluloflavus*
l-Arabinose	++	++
Aesculin	+	
Dulcitol	—	—
d-Galactose	+	++
Glycerol	+++	
Glucose	+++	+++
Lactose	+	+++
Levulose	+	
Maltose	++	+++
Mannose	—	
dl-Mannitol	+	+
Raffinose	+	+++
Rhamnose	++	++
Salicin	+	
Soluble starch	+++	+++
Sorbitol	+	—
Sucrose	+	++

— = no growth ; + = poor growth ; ++ = fair or moderate growth ; +++ = good growth.

organisms were streaked. For fungi with yeast type growth, the activity was determined by serial dilution method. The maximum concentration of the antibiotic used was 20 μg/ml. *In vitro* activity of the antibiotic against some of the fungi tested is given in Table III.

TABLE III. ANTIMICROBIAL SPECTRUM OF HAMYCIN

Test organism	Minimum inhibitory concentration, μg/ml.
Alleschaeria boydii ..	< 5.0
Aspergillus Fumigatus ..	>20.0
Aspergillus niger ..	0.125
Candida albicans ..	0.01*
Cephalosporium madurae	>20.0
Circinella umbellata ..	2.5 — 5.0
Cladosporium wernecki ..	5.0
Cunninghmella elegans ..	20.0
Curvularia lunata ..	0.025
Cryptococcus neoformans	0.005*
Ephelis oryzae	< 1.0
Epidermophyton floccosum	5.0 — 10.0
Macrophomina phaseoli	1.0
Microsporum canis ..	10.0 — 15.0
Nocardia madurae ..	20.0
Phialophora jeanselmi ..	< 5.0
Phytophthora parasitica	20.0
Pythium aphanidermatum	5.0
Saccharaomyces cerevisiae	0.012 0.015
Sporotrichum sp. ..	5.0 — 10.0*
Torula sp.	5.0 — 10.0
Trichoderma lignorum ..	1.0 — 5.0
Trichophyton mentagrophytes	20.0
Trichophyton rubrum ..	20.0

*Turbidimetric assay.

From the above data it is evident that hamycin is very active against fungi with yeast-like growth such as *Candida albicans*, *Cryptococcus neoformans*, *Saccharomyces cerevisiae*, etc. Among the filamentous fungi tested *Aspergillus niger* and *Curvularia lunata* are most sensitive, the minimum inhibitory concentration being 0.125 and 0.025 μg/ml. respectively.

The *in vitro* activity of hamycin was compared with that of two other polyene antifungal antibiotics *viz.* nystatin (Squibb) and antibiotic PA-150 (obtained through the kind courtesy of Messrs. Chas. Pfizer and Co., New York). Three test organisms were used and the results are presented in Table IV.

TABLE IV. COMPARISON OF *IN VITRO* ACTIVITY OF NYSTATIN, HAMYCIN AND PA-150

Antibiotic	Minimum inhibitory concentration, μg/ml.		
	C. albicans	*S. cerevisiae*	*A. niger*
Hamycin	0.01	0.012	0.125
Nystatin	1.5	1.0	2.0
PA-150	0.1	0.15-0.20	10.0

From Table IV it is evident that the three test organisms are more sensitive to hamycin than to nystatin or PA-150. Against *Candida albicans* and *S. cerevisiae* the *in vitro* activity of hamycin is almost hundred times that of nystatin and ten times that of PA-150.

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Hamycin, a New Antifungal Antibiotic

II. ISOLATION AND CHEMICAL PROPERTIES

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STREPTOMYCES pimprina Thirum.[1,2] a new species of *Streptomyces*, has been found to produce at least five antibiotics. The filtered broth contains four of the antibiotics, three of which have been characterized as thiolutin[3,5], aureothricin[3, 4, 5] and *iso*-butyropyrrothine[6]. The natural occurrence of the latter has not been reported earlier. The fourth antibiotic does not belong to the thiolutin group but is yet to be completely characterized. The fifth antibiotic, isolated from the mycelium, is a polyene (heptaene), with very high activity against *candida albicans* (0.01 μg/ml.) but has no activity against bacteria. This antifungal heptaene is named hamycin, and methods for its assay have been reported earlier[7]. In the present paper the isolation and purification and some physico-chemical properties of hamycin are described.

ISOLATION AND PURIFICATION

Method I

Streptomyces pimprina Thirum. was grown in a suitable nutrient medium [1, 2] and the fermentation broth (2,000 l.), harvested after 120 hr. growth at 28°, was filtered. The pH was about 6.8. The filtrate was separately worked up to isolate the thiolutin group of antibiotics.[6] The mycelial cake was washed with water and extracted with *n*-butanol (400 l.). After separating the butanol extract by filtration the cake was again extracted with moist *n*-butanol (250 l.). The butanol extracts were pooled, washed twice with water and concentrated to about 70 l. under reduced pressure below 50°, to give a yellowish suspension. The suspension was cooled to room temperature (28°), filtered, and the yellowish cake obtained was successively washed with *n*-butanol, chloroform (3 l.), benzene (3 l.) and finally with absolute alcohol (1.5 l.), slurrying each time in the solvent and filtering. The washed material was dried first in air and then under high vacuum (1 mm.) over calcium chloride to give 320 g. of crude hamycin.

For purification, pulverized hamycin (10 g.) was added to hot 60% ethanol (5 l.) under stirring. Heating was continued for 20 min. when a clear solution was obtained. Activated charcoal (0.5 g.) and supercel (0.5 g.) were added, and after 5 min. the solution was filtered hot. The filtrate on cooling to 5° gave golden yellow, amorphous precipitate, which was separated by filtration, washed with absolute alcohol and dried under high vacuum (1 mm.) to give 4.6 g. of pure golden yellow hamycin. Concentration of the filtrate gave another crop of 2.5 g. Inhibition concentration of the pure compound was 0.0125 to 0.01 μg/ml. against *C. albicans*.

Method II

The mycelial cake obtained on filtering the fermentation broth was washed with water and dried as far as possible on the filter press itself by passing compressed air. The dried mycelial cake (6 kg.) was extracted with 13% methanolic calcium chloride (8 l.) by stirring, and the solution filtered.

The cake was again extracted with methanolic calcium chloride (8 l.) The methanolic filtrates were combined, clarified with activated charocal, and concentrated under reduced pressure below 45° to about one-third the original volume. The concentrate was diluted with 4 vol. of water and cooled. The precipitated crude hamycin was filtered and washed successively with water, 95% ethyl alcohol, chloroform, benzene and finally with absolute alcohol, to give 12 g. of crude hamycin. The crude product may be purified with 60% ethanol as mentioned under method I.

The crude product obtained from butanol extract as in method I can also be purified with methanolic calcium chloride to give a compound with an activity of 1 part in 80 million to 100 million against *C. albicans*.

PHYSICO-CHEMICAL PROPERTIES

The physical and chemical properties of hamycin are summarized in Table I. The material used for these studies was obtained by purifying crude hamycin (Method I) with 60% ethanol three times, the first crop alone being taken for each successive purification.

From the ultraviolet absorption spectrum it is apparent that hamycin is a polyene (heptaene). Hamycin has a higher *in vitro* activity against *C. albicans* than other heptaenes reported so far. Hamycin is amphoteric and can, therefore, be distinguished from the antifungal heptaenes AYF-A[8], AYF-B[8], antibiotic 757[9], and antibiotic 26/1[10], which are all acidic.

The well characterized amphoteric heptaenes are ascosin[11], candicidin[12, 13], candidin[14], trichomycin A,B[15, 16], amphotericin B[17, 18] and PA-150[9, 18, 19]. Of these two broad groups can be distinguished based on the ultraviolet absorption spectrum. The ascosin-trichomycins-candicidin-PA 150 group is characterized by absorption maxima at 358, 377 and 399 mμ

Table I. Physico-Chemical Properties of Hamycin

Colour	Golden yellow, amorphous powder
Chemical nature	Amphoteric
Melting point	No definite melting point ; decomposes slowly above 160°
Analysis	Found: C, 58.2 ; H, 8.3 ; N, 2.1 %. Sulphur and halogens absent.
Optical rotation	$[\alpha]_D^{25°}$ +216 (c, 0.2% pyridine)
Ultraviolet absorption in 80% methanol (Fig. 1)	λ_{max} in mμ: 345, 363, 383, 406 ; $E_{1cm}^{1\%}$: 413, 635, 916, 875
Major infrared bands (Fig. 2)	3.0, 3.45, 4.35, 5.8, 6.1, 6.3, 6.85, 7.25, 8.5, 8.83, 9.35, 9.6, 9.96, 11.8μ.
Solubility at room temp. (28°) (mg./ml.)	60% methanol, 0.54 ; 60% ethanol, 2.06 ; 50% *n*-propanol, 3·08 50% *iso*-propanol, 3.22. Soluble in pyridine, and dimethylformamide.
Conc. H_2SO_4 reaction	Deep blue

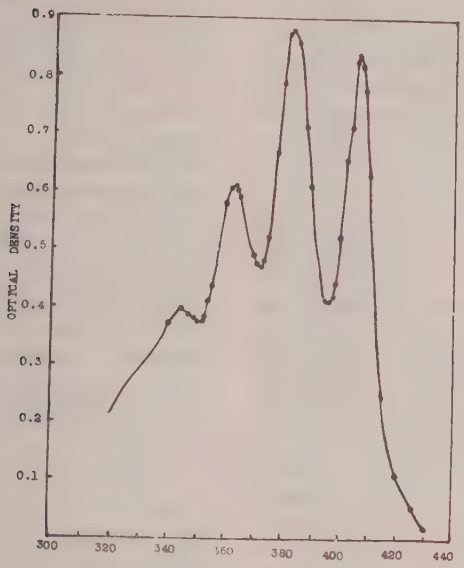

Fig. 1. Absorption spectrum of hamycin (9·6 μg/ml. in 80% methanol)

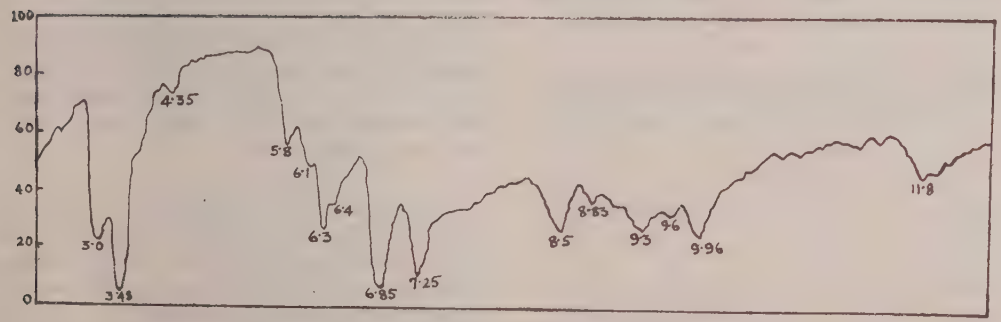

Fig. 2. Infrared spectrum of hamycin (in nujol)

while the candidin-amphotericin B group has absorption maxima at 363, 383 and 406 mμ. Hamycin belongs to the latter group.

Microanalytical data of hamycin and other amphoteric antifungal heptaenes are given in Table II.

Table II. Analysis of Hamycin and other Amphoteric Heptaene Antibiotics.

Heptaene	Analysis (%)		
	C	H	N
Amphotericin B[17] ..	57.59	8.00	1.70
PA-150[19]	62.03	7.83	2.73
Trichomycin A[15, 16] ..	59.49	8.09	2.16
Trichomycin B[15, 16] ..	59.14	8.25	2.24
Candidin[14]	60.20	8.24	1.52
Hamycin	58.20	8.30	2.10

May, 1961

From the analytical data, hamycin resembles amphotericin B ; but hamycin is readily soluble in methanolic calcium chloride, while amphotericin B is insoluble and is actually separated from amphotericin A on the basis of this property.

Hamycin (10 mg.) was hydrolysed with freshly distilled 6 *N* HCl (3 ml.) in a sealed tube at 105° for 16 hr. The hydrolysate was cooled, centrifuged and the supernatent evaporated to dryness. The residue was dissolved in a drop or two of water, placed as a single spot on chromatographic paper (Whatman No. 1), and developed with an upper phase of *n*-butanol-acetic acid-water (40:10:50) for 16 hr. at 25° by descending technique. After drying the paper at room temperature (28°), it was sprayed with 0.1% solution of ninhydrin in *n*-butanol, and on heating at 100° for 10 min. nine ninhydrin positive spots developed. By running marker amino acids on the same paper the ninhydrin positive spots were identified as nine amino acids given in Table III.

Table III. Chromatography of Hamycin Hydrolysate (Developing Solvent, Upper Phase of *n*-Butanol-Acetic Acit-Water (40:10:50)

R_F	Amino acid
0.12	Lysine
0.14	Arginine
0.20	Aspartic acid
0.28	Glutamic acid
0.37	Alanine
0.47	Tyrosine
0.56	Valine
0.64	Phenylalanine
0.70	Leucine

The D and L forms of the amino acids were not distinguished. The nitrogen function in the hamycin molecule appears to be derived from amino acids of a peptide portion. Since hamycin may contain a peptide made up of at least nine amino acids, the molecule will contain at least nine nitrogen atoms even if it is assumed that the peptide is made up of one residue of each of the amino acids. The total nitrogen in the antibiotic being about 2.0 per cent, the molecular weight would be of the order of several thousands. The solubility properties, amorphous nature and indiffusibility in agar also indicate the very high molecular weight of hamycin.

DISCUSSION

Some of the polyene antibiotics have been reported to be ninhydrin positive, but the exact function of the nitrogen[20] is not known except in the case of nystatin, amphotericin B and pimaricin[21]. In these the nitrogen is present as the amino-sugar mycosamine. In trichomycin the nitrogen exists as p-aminophenyl substituent. In the other nitrogen containing polyenes it has been suggested, on the basis of microanalysis or equivalent weight, that the molecule consists of either one or two nitrogen atoms only.

In an earlier communication[22] one of us (D. S. B.) reported that pumilin, a polyene (heptaene) obtained from a strain belonging to the *Bacillus subtilis-pumilus* group, also contains a peptide[23] as a portion of the molecule and several amino acids were identified in pumilin hydrolysate. Pumilin, though a polyene, is essentially an antibacterial antibiotic.

Experiments carried out with hydrolysates of nystatin, amphotericin B, PA-150, candidin and etruscomycin indicate that these polyenes also may contain peptide portions in their molecules. However, the relative proportions of different amino acids seem to differ in different polyenes. Details

of the work on the amino acid contents of some of the antifungal polyenes will be published elsewhere.

SUMMARY

The isolation and characterization of hamycin, a new antifungal heptaene, produced by *Streptomyces pimprina* Thirum., are described. Hamycin is differentiated from the known antifungal heptaenes on the basis of physical, chemical and biological properties. The nitrogen function in hamycin molecule is briefly discussed.

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Studies on the Feed Rate for Precursor and Sugar in Penicillin Fermentation*

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Induced mutation and selection are the most widely used methods for developing high yielding strains of *Penicillium chrysogenum*. The main criterion for selection is the increasing ability of the strains to synthesise penicillin. With this advance in one particular function of the mould its demands on fermentation environment vary and become more specialized and exacting than the parent strains. It is, therefore, necessary to establish the optimum fermentation conditions for each of these strains before they can be used commercially.

In the classical cornsteep-lactose medium for penicillin fermentation developed by Moyer and Coghill,[1] lactose is incorporated initially in the medium and because the mould *P. chrysogenum* utilizes this sugar at a slow rate the optimum condition with regard to growth and pH of the fermentation medium could be maintained at the desired level. In recent years lactose has been successfully replaced with cheaper and more readily available sugars like sucrose, glucose and molasses.[2-7] However, on account of their faster utilization by the mould, these sugars have to be fed in regulated doses throughout the fermentation.

Compounds like phenylacetic acid are known to be toxic to living cells. From an analogy of detoxication of phenylacetic acid in animals, one is tempted to speculate that production of penicillin G by *P. chrysogenum* and other moulds is one of the complex mechanisms of detoxication of phenylacetic acid in these organisms. The oxidation rate of externally fed phenylacetic acid by the mould mycelia of high yielding mutants is found to be very low or negligible as compared to the high rate of oxidation in low yielding parent strains.[8] It, therefore, seems possible that the detoxication of phenylacetic acid in high yielding strains of *P. chrysogenum* is mainly through formation of the complex penicillin molecule rather than through oxidation. In commercial penicillin fermentation with high yielding strains over 90 per cent of the added precursor is actually found to be incorporated into the penicillin molecule.

In view of the crucial importance of the optimum feed-rates of both sugar and precursor in penicillin fermentation and as they vary from strain to strain, the present work was undertaken to determine the optimum feed-rates of these two media constituents for maximum penicillin production by a commercial strain of *P. chrysogenum* HA-6, and also to investigate the possibility of prolonging the penicillin biosynthetic phase in the fermentation by feeding a suitable mixture of precursor and sugars for an overall increase in penicillin yield.

MATERIALS AND METHODS

Strain: ¶ *Penicillium chrysogenum* HA-6, developed from Russian "New Hybrid".

* Forming part of a thesis submitted by K. Chaturbhuj for the degree of Master of Science of Bombay University.

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Media

1. Sporulating Medium :

The sporulating agar medium employed for developing spores from soil stock culture contained glycerine, molasses, yeast extract and minerals.

2. Seed Medium :

The seed medium used for developing the vegetative inoculum from the spores contained :

	g/l.
Cornsteep liquor	30
Sucrose	20
$NaNO_3$	2.75
$CaCO_3$	3.0
KH_2PO_4	0.25
$MgSO_4.7H_2O$	0.06

pH was adjusted to 5.5 before sterilization.

3. Fermentation Media :

Medium A contained :

	g/l.
Lactose	40
Peanut meal	30
Cornsteep liquor	5
$CaCO_3$	5
Phenylacetamide	0.5
Na_2SO_4	1.3
$MgSO_4.7H_2O$	0.06

pH was adjusted to 6.6 before sterilization. ¶Medium B: Same as Medium A but contained 3 g. sucrose in place of 40 g. lactose.

Fermentation technique

All media were steam sterilized for 30 min. at 120° and 15 lb. pressure.

Ten to twenty million spores from agar slants were used to inoculate 100 ml. of seed medium in 500 ml. Erlenmeyer flasks. Ten ml. of 48 hr. old vegetative inoculum were used to inoculate 90 ml. of fermentation medium in 500 ml. flasks. The development of the vegetative inoculum and the production of penicillin were carried out at 24° on a rotary shaker, 240 r.p.m., 2″ throw. All fermentations were carried out in duplicate.

Analytical methods

Penicillin was estimated by a modified iodometric method,[10] and pH was determined by glass electrode. Mycelial dry weights were determined by filtering the mycelium from a 10 ml. sample, washing once with 0.2N HCl and then twice with distilled water and finally drying at 90° for 24 hr. Sucrose and lactose were estimated by the A.O.A.C. method.[11] Mycelial nitrogen was determined by Kjeldahl's method.

RESULTS AND DISCUSSION

Optimum concentration of phenylacetic acid

Initial incorporation of 0.1 per cent solid phenylacetic acid in the fermentation medium gave the highest yield of penicillin with this particular strain (Table I).

With initial phenylacetamide at 0.05 per cent in the medium, feeding additional 0.1 per cent precursor as sodium phenylacetate at pH 7.0 in a single dose or 10 equal doses at 12 hr. intervals beginning from 24 hr. after inoluation, showed practically no difference in penicillin titre. Higher concentrations of precursor (0.5 per cent and above) fed in divided doses inhibited penicillin production.

On the basis of these results several fermentation trials were made in the factory fermentors, by adding solid phenylacetic acid in the medium before sterilization. Penicillin titres were not only as good as in fermentation with divided doses of precursor (as solution of sodium salt) added at intervals during fermentation, but were also more uniform from batch to batch. The operational advantage of incorporating the entire amount of precursor in the medium before sterilization is obvious.

TABLE I. EFFECT OF DIFFERENT CONCENTRATIONS OF PHENYLACETIC ACID, IN SINGLE OR DIVIDED DOSES, ON PENICILLIN YIELD

Initial phenylacetamide %	Intial phenylacetic acid %	Additional phenylacetic acid, fed during fermentation %	At 144 hr.	
			pH	Penicillin u/ml.
0.05	Nil	0.1*	7.4	2,880
0.05	Nil	0.1**	7.4	2,710
0.05	Nil	0.25**	7.4	2,820
0.05	Nil	0.5**	7.6	2,220
0.05	Nil	1.0**	7.6	2,110
0.05	0.1	Nil	7.2	2,380
Nil	0.1	Nil	7.1	3,220
Nil	0.2	Nil	7.1	2,460
Nil.	0.3	Nil	7.1	2,300

* Added in a single dose, 24 hr. after inoculation of the fermentation medium.

** Added in 10 equal doses at 12 hr. intervals, beginning from 24 hr. after inoculation.

Prolongation of the phase of penicillin biosynthesis by sucrose-precursor mixture feeding

Preliminary studies indicated that if a mixture of sucrose and precursor was fed intermittently, mycelial autolysis was delayed considerably and pH was maintained within a favourable range for a longer period. There was also economy in the total consumption of both the ingredients. Success in this respect was reported earlier by Hosler and Johnson[5] in laboratory fermentors. But in their studies no attempt was made to prolong the phase of penicillin biosynthesis by feeding such mixtures. With the strain Q-176, and at the feed-rate of 0.0042 per cent per hour of potassium phenylacetate the optimum glucose feed-rate was found to be 0.035 per cent per hour in a synthetic medium.

In shake flask fermentation with lactose, mycelial autolysis usually sets in at about 120 hr., and pH rises to an unfavourable region of about 8.0. Maximum penicillin titre obtainable is around 2,700 u/ml. Using medium B, with a sucrose feed-rate of 0.031 per cent per hour (found to be the optimum) and precursor feed-rate of 0.0008 per cent per hour, fermentation was prolonged to 264 hr. with a much higher penicillin yield (3,250 u/ml.). The pH was also maintained in the favourable range (Table II). These rates of feeding of the mixture were found to be the optimum from subsequent studies with different precursor feed-rates. Maximum penicillin titre obtained in one experiment was 3,700 u/ml. at 312 hr. It must, however, be mentioned that the volume of the fermentation medium was gradually depleted due to withdrawal of samples for analysis resulting in a probable increase in aeration efficiency. The final volume of the medium at the end of 312 hr. was 60 ml.

Sucrose-precursor mixture feeding in lactose medium

Using 2 per cent lactose instead of 4 per cent in Medium A, the phase of penicillin

TABLE II : PROLONGATION OF THE PHASE OF PENICILLIN BIOSYNTHESIS BY SUCROSE PRECURSOR MIXTURE FEEDING

Sucrose %/hr.	0.02		0.031		0.05		0.07	
Age in hr.	pH	Penicillin u/ml.	pH	Penicillin u/ml.	pH	Penicillin u/ml.	pH	Penicillin u/ml.
120	7.5	730	6.9	1,240	6.9	1,110	6.9	1,080
144	7.8	800	7.1	1,500	7.1	1,200	7.1	1,200
168	8.2	200	7.2	1,900	7.2	1,340	7.2	1,320
192			7.1	2,400	7.1	1,450	7.1	1,450
216			7.1	2,650	7.1	1,500	7.1	1,500
240			7.1	3,000	6.9	1,630	6.9	1,630
264			7.1	3,250	6.9	1,600	6.9	1,600

Precursor feed-rate was kept constant and corresponding to 0.0008 %/hr. Feedings were made at 12 hr. intervals.

biosynthesis was also prolonged by feeding sucrose-precursor mixture from 72 hr. onwards by which time the initial lactose was practically used up. Best result was again obtained with the precursor feed-rate of 0.0008 per cent per hour and sucrose feed-rate of 0.031 per cent per hour (Table III); fermentation was prolonged to 240 hr. with a final penicillin yield of 4,250 u/ml. On the basis of these laboratory results, it had actually been possible to obtain higher yields of penicillin, as compared to penicillin yield with 4.0 per cent lactose medium, in the factory fermentors using 2.0 per cent lactose initially and feeding suitable concentration of sucrose intermittently from 48 hr. while precursor was fed separately.

Chemical changes in sucrose-presursor mixture fed fermentation

The comparative biochemical pictures of sucrose-precursor mixture fed fermentations and normal lactose medium fermentation are shown in Fig. 1 to 3. Four

TABLE III : SUCROSE-PRECURSOR MIXTURE FEEDING IN FERMENTATIONS STARTED WITH 2.0% LACTOSE

Precursor %/hr.	0.0008		0.0021		0.0042		Control	
Age in hr.	pH	Penicillin u/ml.	pH	Penicillin u/ml.	pH	Penicillin u/ml.	pH	Penicillin u/ml.
120	7.8	1,980	7.8	1,980	7.8	1,900	7.8	2,770
144	8.0	2,060	8.2	1,930	8.2	1,960	8.5	2,030
168	8.0	2,640	8.2	2,640	8.2	2,480		
192	8.0	3,050	8.0	3,030	8.1	2,590		
216	7.6	3,650	7.8	3,140	7.9	2,640		
240	7.4	4,250	7.5	3,460	7.6	3,040		

Sucrose feed-rate was kept constant corresponding to 0.031 per cent. Feedings were made every 12 hr. 4.0 per cent lactose medium (Medium A) with 0.1 per cent phenylacetic acid initially.

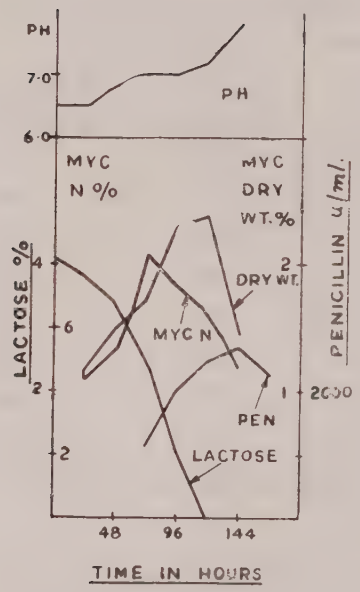

Fig. 1.
Medium A

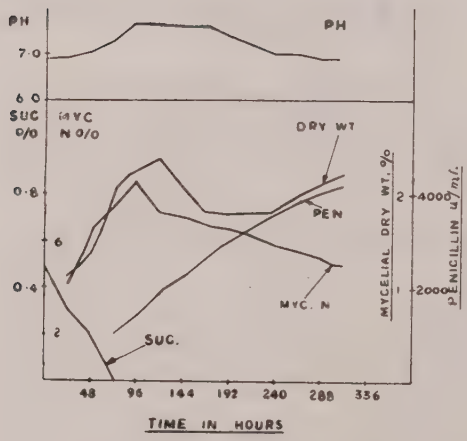

Fig. 2.
Medium B, 0.3% initial sucrose

replicate flasks were run in each experiment and not more than four withdrawals (10 ml. every 24 hr.) were made from any of the flasks. Final volume in all the flasks was about 70 ml.

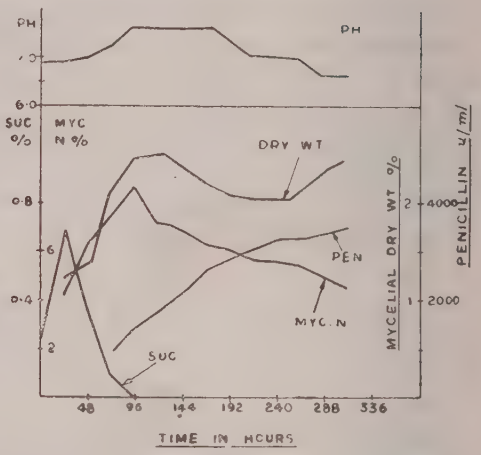

Fig. 3.
Medium B-3, No initial sucrose

In contrast to gradual slowing down and complete cessation of penicillin biosynthesis after 144 hr. in the controls (Fig. 1) penicillin production in the sucrose-precursor mixture fed fermentations (Fig. 2 and 3) went on with approximately uniform rate throughout the greater part of this prolonged fermentation over 300 hr. When feeding was started from the very beginning (Fig. 3, no initial sucrose) the penicillin yield was slightly lower as compared to fermentation with 0.3 per cent initial sucrose (Fig. 2). This appeared to be correlated with the mycelial nitrogen content which was maintained at a lower level in the former (Fig. 3).

With sucrose-precursor mixture feeding, mycelial build-up in respect of dry weight and nitrogen during the early stages (upto 96 hr.), was not very much different from that in the control fermentation. However, although both dry weight and mycelial nitrogen content slightly declined later, the values were more or less steady upto 240 hr. An interesting aspect of the sucrose-precursor mixture fed fermentation was that the mycelial dry weight began to increase again after 240 hr. as a result of mycelial regeneration but the mycelial nitrogen content was not commensurate

with the increase in dry weight. This perhaps explains why the rate of penicillin biosynthesis gradually declined during the later stages (after 240 hr.). This decline is particularly noticeable in Fig 3.

Since with sucrose-precursor mixture feeding the fermentation tended to flatten after 240 hr., it seemed likely that nitrogen was becoming limiting, autolysed mycelium not supplying the nitrogen in the form in which it could be readily assimilated by the mould. Nitrogen supplementation along with sucrose-precursor mixture feeding might be helpful in maintaining the maximum rate of penicillin biosynthesis for a longer period with the ultimate increase in the penicillin yield. Further work is warranted in this direction.

Sucrose-precursor mixture feeding on samples from large fermentors

Samples of mycelial suspensions (100 ml.) were collected aseptically in 500 ml. flasks from factory fermentors at different stages of fermentation and the fermentations were continued on the shaker with sucrose-precursor mixture feeding. In the first set of experiments a 72 hr. sample was drawn from a sucrose fed fermentation. In the second and third sets, samples were drawn from lactose medium fermentations respectively at 72 hr. when there was only 0.2 per cent residual lactose and at 80 hr. when all the lactose was exhausted. The results of these experiments are presented in Table IV. In all these experiments, control fermentations were carried out without any mixture feeding except in experiment 1, where only sucrose was fed at the rate of 0.031 per cent per hour in the control.

In the fermentors precursor addition was completed before 72 hr. The controls, therefore, served as reference for any improvement achieved by sucrose-precursor mixture feeding. It must be mentioned that by changing the fermentation environment

TABLE IV. SUCROSE-PRECURSOR MIXTURE FEEDING[1] ON SAMPLES FROM LARGE FERMENTORS

Experiment No.			Age in hours					
			72	96	120	144	168	192
I	Control[2] (Sucrose-feeding 0.031 % 1 hr.)	pH	7.8	7.6	7.1	6.7	6.4	
		Pen. u/ml.	2,480	2,670	2,240	1,920	1,640	
	Mixture feeding	pH		7.8	7.8	7.8	7.8	7.6
		Pen. u/ml.		2,900	3,170	3,760	3,960	4,300
II[3]	Control (No feeding)	pH	7.5	8.0	8.2	8.4		
		Pen. u/ml.	2,740	2,740	2,260	1,800		
	Mixture feeding	pH		7.7	7.8	7.8	7.8	
		Pen. u/ml.		3,090	3,140	3,670	4,000	
III[3]	Control (No feeding)	pH	7.2	7.5	8.0	8.4		
		Pen. u/ml.	3,200	2,960	2,960	2,240		
	Mixture feeding	pH		7.2	7.2	7.3	7.2	
		Pen. u/ml.		3,190	3,440	2,750	4,360	

1. Sucrose-precursor mixture added every 12 hr. at the rates corresponding to 0.031 and 0.0008 per cent per hour respectively.
2. Sucrose solution added every 12 hr. at a rate corresponding to 0.031 per cent per hr.
3. Samples withdrawn from fermentor at 80 hr. age.

from highly agitated and aerated condition in the fermentor to the shaken flask, the rate of penicillin formation was considerably reduced in the controls. The maximum penicillin titre was only 2,670 u/ml. at 96 hr. Thereafter pH gradually became unfavourable with a consequent loss of penicillin. But the parallel samples when fed with surcose-precursor mixtures, continued to produce penicillin for a longer period and gave maximum titres of 4,300, 4,000 and 4,360 u/ml. respectively in the three sets of experiments.

The above results clearly indicate the possibility of adopting the sucrose-precursor mixture feeding technique in the plant in surcrose as well as lactose medium for higher final yields in possibly longer cycles. The optimum feed-rates under plant conditions in agitated and aerated fermentors have, of course, to be empirically established. The shaken flasks results could be used as a guide.

Penicillin production with sucrose-precursor mixture feeding in 500 gallon fermentor

The results of experiments actually carried out in a 500 gallon agitated and aerated fermentor in this respect are presented in Table V. In the lactose control 0.1 per cent phenylacetic acid was fed at regular intervals in divided doses. In the sucrose-precursor mixture fed tank Medium B was used and the mixture was fed at hourly intervals at the rate of 0.033 per cent sucrose and 0.0015 per cent precursor per hour from 24 hr. onwards.

In the control fermentation with lactose the maximum titre of 3,440 u/ml. was obtained at 112 hr. There was no further increase in the titre even after prolonging the fermentation. With sucrose-precursor mixture feeding the fermentation was prolonged to 192 hr. with a final titre as high as 4,700 u/ml. The pH was maintained very effectively within the favourable range. Under similar conditions of fermentation, if sucrose and precursor are fed separately the maximum titre obtainable is usually about 3,500 u/ml (data not shown), the feed-rate of sucrose being much higher. The consumption of precursor within the same period is also comparatively higher if it is fed separately at longer intervals.

The above results thus established the following facts. In plant fermentation practice using sucrose, raw material economy in both sucrose and precursor can be effected by feeding these two ingredients as a mixture. It is also possible

Table V. Penicillin Production with Sucrose-Precursor Mixture Feeding in 500 Gallon Fermentor

A : Lactose control			B: Sucrose Mixture — Precursor Feeding		
Age in hr.	pH	Penicillin u/ml.	Age in hr.	pH	Penicillin u/ml.
42	6.8	1,000	41	7.0	750
52	6.9	1,400	65	7.0	1,450
76	7.0	2,300	89	7.0	2,600
90	7.0	3,150	113	7.0	3,500
112	7.1	3,440	158	7.3	4,500
120	7.2	3,440	180	7.0	4,650
			192	6.7	4,700

by this mixture feeding technique to maintain the physiological condition of the mycelium optimal for penicillin biosynthesis for a much longer period with an ultimate increase in penicillin yield. If the optimum proportions and feed-rates could be established for a given fermentation environment in highly agitated and aerated fermentors, and if the highest rate of penicillin biosynthesis could be maintained for a longer period, it would be economical even if the batch is of longer duration. The present studies on sucrose-precursor mixture feeding strongly indicate such a possibility in industrial penicillin fermentation.

SUMMARY

The optimum feed level of precursor (phenylacetic acid) for penicillin production by *Penicillium chrysogenum* HA-6, was found to be 0.1 per cent incorporated in the medium in one lot before sterilization.

The phase of penicillin biosynthesis was prolonged beyond 12 days in shaken flasks, instead of the usual 5 to 6 days, by feeding every 12 hr. a mixture of sucrose and precursor at the rates corresponding to 0.031 per cent and 0.0008 per cent per hour respectively.

Pilot scale fermentation in 500 gallon fermentors gave a penicillin titre of 4,700 u/ml. by sucrose-precursor mixture feeding as compared to 3,500 u/ml. in the control.

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Physico-Chemical Data on Antibiotics

II. ANTIBIOTICS PRODUCED BY *ACTINOMYCETES*

2. Antibiotics with antitumour activity

MAJORITY of the antitumour antibiotics reported so far have been isolated from culture filtrates of *Streptomyces* species. A few compounds isolated from cultures of other groups of microorganisms which have activity against tumours, are listed in Table I.

Selected physical, chemical and biological properties of antitumour antibiotics from actinomycetes are summarized in Table II, continuing the serial numbering from the previous compilation.[1] Compounds listed in the earlier series are cross-referred to with additional information, if any. The compounds are grouped as follows :

Aminoacid and polypeptide type ;
other ninhydrin positive and probably polypeptide compounds ;
diazoketones ;
nitrogen heterocyclics ;
alicyclic carboxylic acids ;
cycloheximide-type ;
quinonoidal compounds ;
aliphatic acid amides ;
polyenes ; and
miscellaneous compounds.

Melting point, ultraviolet absorption maxima and empirical formulae indices are provided as in previous compilations of this series.

In addition to the usual abbreviations the following have been used in Table II :

DMF *for* dimethylformamide
DMS *for* dimethylsulfoxide
2,4-DNPH *for* 2,4-dinitrophenylhydrazine

An aesterick (*) indicates that the complete or partial structure of the compound is known.

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TABLE I. ANTITUMOUR ANTIBIOTICS FROM MOULDS, FUNGI & BACTERIA

Producing organism	Antibiotic factor	Reference
FUNGI		
BASIDIOMYCETES		
Boletus edulis	Factor	*Antibiot. and Chemother.* **7**, 1 (1957)
Clitopilus abortivus	Factor	
Collybia radicata var. *furfuracea*	Factor	
Calvatia spp.	Factors	*Antibiot. Ann.* (1958-59) 493. *Science* **132**, 1897 (1913).
Clitocybe nebularis	Nebularine	*H. T.* **2.**
FUNG IMPERFECTII		
Aspergillus fumigatus, and other fungi	Gliotoxin	*H. T.* **2.**
Aspergillus fumigatus, *Aspergillus* spp., *Penicillium* spp.	Fumagillin	*H. T.* **2.**
Fusarium sporotrichella var. *poae* Bilai	Poin	*Antibiotics* (*Trans.*) **4**, 431 (1959).
Helminthosporium sp.	Factor	
BACTERIA		
Acetobacter aceti	Factor	*Nature* **185**, 176 (1960).

1. *Hindustan Antibiot. Bull.* **2**, 131 (1960).

TABLE II. ANTITUMOUR ANTIBIOTICS PRODUCED BY *ACTINOMYCETES*

S. No.	Antibiotic, and Producing organism	Chem. nature. Mol. composition	M. p.	λ_{max} in mμ	$(\alpha)_D$	Colour reactions, functional group tests.	Antibiotic & Antitumour activity. References
1	2	3	4	5	6	7	8
			AMINOACID AND POLYPEPTIDE TYPE				
153	*AZASERINE *S. fragilis* By synthesis	Light yellow-green needles. *o*-Diazoacetyl-L-serine. $C_5H_7N_3O_4$	142-62 dec.	250.5 (pH 7) 252 (0.1N NaOH)	—0.5 (H_2O)		Active against Crocker mouse sarcoma 180, Murphy-Sturm lymphosarcoma and Walker carcinoma in rats; adenocarcinoma E-0771, etc. Moderate activity against some bacteria, fungi protozoa. *H T.* **2.**
154	*6-DIAZO-5-OXO-L-NORLEUCINE (DON) *Streptomyces sp.*	Light yellow-green cryst. pk_a 2.1 and 8.95 in H_2O. $C_6H_9N_3O_3$	145-55 dec.	244, 274 (phosphate buffer pH 7)	+21 (H_2O)	Analogue of azaserine (153)	Crocker sarcoma 180 inhibited. Moderate activity against some bacteria, fungi, protozoa. *Antibiotic. and Chemother.* **6,** 487 (1956); *ibid.* **7,** 81 (1957); *J. Am. Chem. Soc.* **78,** 3075 (1956); *ibid.* **80,** 3941 (1958); *Chem. Ber.* **91,** 1037 (1958).
155	ALAZOPEPTIN *S. griseoplanus*	Contains one mole α-alanine in a peptide with 2 moles of a C_6 diazoketo-amino acid, that is oxidizable to glutamic acid. $C_{15}H_{21}N_7O_6$		242, 274 (phosphate buffer pH 7)	+9.5 (H_2O)		Sarcoma 180 in mice. Also active against some bacteria. *Antibiot. Ann.* (1956-57) 730

1	2	3	4	5	6	7	8
156	CARCINOCIDIN *S. kitazawaensis.* *Streptomyces* sp.	Brownish black powder. Polypeptide with S; gives cystine, lysine, glycine, glutamic acid on hydrolysis	360 dec.		−20 (H_2O)	Pos. xanthoproteic, Pauli; neg. biuret, Adamkiewicz, conc. H_2SO_4, Liebermann, ninhydrin, diphenylamine, cysteine-H_2SO_4, nitroprusside, Folin, anthrone, Sakaguchi, $FeCl_3$; pseudopos. Millon.	Ehrlich carcinoma, Yoshida rat sarcoma, respond. *C. albicans T. utilis, Sacc. cerevisiae* inhibited. *H. T.* **2.** *C.A.* **54,** 832f (1960)
157	CARCINOMYCIN (GANMYCIN) *S. carcinomycicus.* *S. ganmycicus*	Dark green compd. Polypeptide-like, without S.	Dec. 280			H_2O insol. ppt. with picric, phosphotungstic, Reinecke's acids. Pos. ninhydrin on hydrolysis. Aq. soln. pos. Sakaguchi, Pauli, Millon, Molisch, naphthoresorcinol; neg. biuret, ninhydrin, xanthoproteic, Liebermann, Adamkiewicz, Na nitroprusside, $FeCl_3$.	Tumours and bacteria inhibited. *C. A.* **54,** 831g (1960); J. *Antibiot. (Jap.)* **8A,** 97, 113 (1956); *ibid* **9B,** 160 (1956)
158	ACTININ *Streptomyces* sp. (*Streptothrix felis*)	Basic polypeptide					*C.A.* **51,** 16894f (1957).
159	ACTINOIDIN *Proactinomyces actinoides* (*Nocardia actinoides*)	White amorphous HCl Polypeptide with 7% N, 2% amino N.				Pos. Pauli, Molisch, biuret. Can be diazotized and in this form gives brown green colour with 1-naphthol. Neg. $FeCl_3$, Fehling, Seliwanoff, amino sugars. Heating with 5% HCl for 3-5 min. gives pos. Fehling and 2 fractions, one insol. in 5% HCl and giving pos. Pauli; HCl sol. fraction is a peptide.	*Antibiotiki* **2,** No. 5, 44 (1957); *ibid.* **3,** No. 1, 51 (1958).

1	2	3	4	5	6	7	8
160	ACTINOLEUKIN *S. aureus*	White platelets or needles. $C_{29}H_{42}N_6O_7S$ or $C_{30}H_{42}N_6O_8S$ or $C_{29}H_{40}N_6O_7S$	213 dec. 191-92 dec.	243, 312	—302 (ethyl acetate)	Neg. ninhydrin, but pos. after hydrolysis, Neg. biuret, Tollens, Fehling, Sakaguchi, Pos. pine splint (green), Ehrlich (yellow), Molisch (purple ring).	Weak antitumour actitity. Inhibits some gram + organisms *H. T.* **2.** *J. Antibiot.* (*Jap.*) **11A,** 160 (1958)
161	*ACTINOMYCIN C *S. chrysomallus*	Brick red bipyramids, prisms, needles. Weakly basic chromopeptide, quinonoid. C_1 contains threonine, sarcosine, proline, valine, *n*-methyl-valine. $C_2 = C_1$ + allo-*iso*-leucine. $C_3 = C_2$ less valine. E_1, E_2 from C-producing strain with DL-*iso*-leucine medium; F_0-F_5 with sarcosine as precursor. C: $C_{60}H_{83}N_{11}O_{16}$ or $C_{62}H_{89}N_{11}O_{17}$ C_3: $C_{64}H_{90}N_{12}O_{16}$	C_1: 241-43 dec. C_2: 237-39 dec. C_3: 232-35 dec.		C_1: —349 ± 10 (MeOH) C_2: —325 + 10 (MeOH) C_3: —321 ± 10 (MeOH)	C gives neg. ninhydrin, grey brown ppt. with Nessler, yellow-green fluorescence in MeOH or CH_3COOH	Cytostatic against sarcoma 180 in mice, RC carcinoma, leukemia L-4946 in mice, Walker carcinoma in rats. Some gram + bacteria inhibited. *H. T.* **2.** *Angew. Chem.* **71,** 194 (1959); *Chem. Ber.* **91,** 779 (1958); *Naturwiss.* **47,** 229, 230 (1960) *Prog. Chem. Org. Nat. Prod* **18,** 1, (1960).
162	AURANTINE *S. aurantiacus*	Dark red hexagonal bipyramids. Actinomycin family closest to Actinomycin C.	251-53				Inhibits gram+bacteria, Ehrlich carcinoma, sarcoma 180, lymphoma in mice; sarcomata, Guerin's carcinoma in rat. *Antibiotics* (*Trans.*) **4,** 428 (1959).

1	2	3	4	5	6	7	8
163	*ACTINOMYCIN D *S. antibioticus*	Bright red rhomboid prisms. Weakly basic chromopeptide, quinonoid. Structure same as for Actinomycin C_3 but allo-*iso*-leucine components replaced by valine. $C_{60}H_{76}N_{12}O_{15}$	241.5; 243 dec.	240, 445 (EtOH); 477 (EtOH-10N HCl); 285, 344, 458 (EtOH-0.1N NaOH); 240-42, 422.	—315 ± 7 (MeOH)		Glioblastoma, adenocarcinoma, melanoma, sarcoma 180, lymphosarcoma. *H. T.* **2.** *J. Chem. Soc.* (1957) 3280. *Prog. Chem. Org. Nat. Prod* **18,** 1 (1960).
164	ACTINOMYCIN J (ACTINOMYCIN 4A-2) *S. flaveolus*	Similar to Actinomycin A. Contains threonine, sarcosine, proline, valine, *n*-methyl-valine.	248-50 dec.	244, 445	—323 (MeOH)		Ehrlich ascites carcinoma in mice, Yoshida rat sarcoma,, Usubuchi rat sarcoma, Takeda rat liver sarcoma, Ridgeway osteogenic sarcoma, ascites tumour. *J. Antibiot. (Jap.)* **9A,** 31 (1956); *ibid.* **10A,** 46, 56 (1957). *H. T.* **2.**
165	ACTINOMYCIN K *S. melanochromogenes*	Red prisms. Contains threonine, sarcosine, proline, valine, isoleucine (?) C 57.72, H 7.57, N 14.06.	250-52	240, 440			*C. A.* **53,** 18383g (1959).
166	ACTINOMYCIN M *Streptomyces* sp. belonging to *S. antibioticus* group.	Red, odourless, hygroscopic, cryst. powder. Contains 3 or 4 fractions. Contains *n*-methyl-valine, L-proline, L-threonine, D-valine, sarcosine. C 57.1, H 6.75, N 12.85.	257 dec.	232-40, 448 (EtOH)	—310 ± 40 (EtOH)	Pos. for quinone	Inhibits gram + bacteria. Antimitotic (radiomimetic) action. *Nature* **179,** 130 (1957); *C. A.* **51,** 4469b (1957).

1	2	3	4	5	6	7	8
167	ROSSIMYCIN *S. chrysomallus*	Brick red cryst. Actinomycin C type. Peptide chain.	254 dec.	450 (acetone, alc. soln).			Gram + organisms. *Myco. phlei*, osteogenic sarcoma. *Indian Phytopath.* **11,** 23 (1958). *Hindustan Antibiot. Bull.* **3,** (1961).
168	MELANOMYCIN *S. melanogenes*	Blackish amorphous powder; amphoteric; melanin-like; non-dialyzable through semi-permeable membrane. Acid hydrolysate contains valine, phenylalanine, leucine, proline, alanine, arginine, histidine, glutamic acid. glycine.		260-70 (HCl or NaOH)		Pos. xanthoproteic, diazo, Na nitroprusside, Millon. Neg. biuret, ninhydrin, Sakaguchi, Fehling. Acid hydrolysate gives pos. Sakaguchi and ninhydrin. Pptd. by $(NH_4)_2SO_4$, trichloroacetic, picric, flavianic, phosphotungstic acids, cupric sulphate, $AgNO_3$, ferric sulphate, $HgCl_3$, potassium alum, sulphosalicylic acid, Pb acetate, $ZnCl_3$, $CaCl_3$, ammonium reineckate. No. ppt. with methyl orange.	Carcinoma in mice. Slight activity against bacteria and fungi. *J. Antibiot.* (*Jap.*) **10A,** 133 (1957); *ibid.* **12A** 150 (1959); *ibid.* **13A,** 172 (1960).
169	BU 306 Actinomycete	Proteinic. Destroyed by organic solvent, and on heating. Activity falls with pH, highest activity at pH 8.					Ehrlich tumour, gram+, gram— bacteria *Folia Biol.* **3,** 263 (1956).
		NINHYDRIN POSITIVE AND PROBABLY POLYPEPTIDE COMPOUNDS					
170	CARDINOPHYLLIN *S. sahachiroi* (*See also* 171, 172)	K salt colourless powder. Contains C, H, N, O.	220 dec. (K salt)	210, 280 (H_2O)		Pos. xanthoproteic, ninhydrin, diphenylamine. Pseudopos. $FeCl_3$, Nessler. Neg. resorcin, Millon, Liebermann.	*C. A.* **52,** 8473d (1958)

1	2	3	4	5	6	7	8
171	CARZINOPHILLIN *S. sahachiroi*			217, 290 (H_2O)		Pos. xanthoproetic. Neg. Molisch, Sakaguchi, Fehling, Benedict, $FeCl_3$, Schiff, Tollens.	Yoshida sarcoma, Ehrlich carcinoma, ascitic hepatoma, sarcoma 180, Krebs carcinoma in mice. Bacteria. actinomycetes inhibited. *H. T.* **2**. *J. Antibiot. (Jap.)* **12B**, 361(1959).
172	CARZINOPHILLIN A *S. sahachiroi*	Colourless needles.	217-22 dec.	218, 250, 283 (MeOH)	+57.8 ($CHCl_3$)	Pos. Baeyer, xanthoproteic, ninhydrin, Br. absorption, 2, 4-DPNH, Neubauer-Rhode, Na-1,2-naphthoquinone-4-sulfonate, Na nitroprusside, diphenylamine, anthrone.	Yoshida sarcoma in rats inhibited. *H. T.* **2**. *J. Antibiot. (Jap.)* **12A**, 144 (1959).
173	DESERTOMYCIN *S. flavofungini*	Snow-white glittering hexagonal cryst. Contains C-methyl group; no acetyl or N-methyl groups. $C_{33}H_{60-62}NO_{14}$	189-90			Pos. ninhydrin; decolourizes $KMnO_4$ or Br.	Cytotoxic to various tumour cells. Gram+ bacteria. *Nature* **182**, 401 (1958)
174	MARINAMYCIN *S. mariensis*	Pale yellow hygroscopic amorphous powder. Not absorbed on active charcoal, Al_2O_3, silica gel, starch, diatomaceous earth, ion exchangers.				Pos. ninhydrin, Sakaguchi, Folin. Neg. indole, diazo, Bial. Ambiguous pos. biuret and Fehling.	Ehrlich ascites and solid tumours. Weak activity against some bacteria. *J. Antibiot. (Jap.)* **12B**, 300 (1959).
				DIAZOKETONES			
175	DIAZOMYCIN A *S. ambofaciens*	Clusters of pale yellow needles. Readily sol. in MeOH. R_F in 80% isopropanol 0.6-0.7. C 40.31, H 5.24, N 17.1; C 44.34, H 5.08, N 17.22 (anhydrous)			275, 245 with $E^{1\%}_{1cm}$ 530, 315 resp.	Light grey blue colour with ninhydrin.	Sarcoma 180, adenocarcinoma 755, and moderate activity against leukemia 1210 *Antibiot. Ann.* (1959-60) 943.

1	2	3	4	5	6	7	8
176	DIAZOMYCIN B *S. ambofaciens*	Sulphur yellow rectangular plates. Slightly sol. in MeOH. R_F in 80% isopropanol, 0.2-0.3. C 43.15, H 5.5, N 19.22.			275, 245 with $E^{1\%}_{1cm}$ 550. 340 resp.	Intensive purple with ninhydrin.	*Antibiot. Ann.* (1959-60) 943.
177	DIAZOMYCIN C *S. ambofaciens*	Cream coloured needles. Readily sol. MeOH. R_F in 80% isopropanol, 0.8-1.0 C 46.03, H 5.93, N 23-12.	Chars c 220		275, 245 with $E^{1\%}_{1cm}$ 340, 210 resp.	Light grey blue colour with ninhydrin.	*Antibiot. Ann.* (1959-60) 943.
		NITROGEN HETEROCYCLIC COMPOUNDS					
178	*GRISEOLUTEIN B *S. griseoluteus*	Yellow prisms. Acidic. 1-Methoxy-4-[(1, 2-dihydroxyethoxy) methyl]-9-carboxy-phenazine. $C_{17}H_{16}N_2O_6$			281-83, 342-44 (MeOH)		Gram+, gram— bacteria, Ehrlich carcinoma inhibited. *H. T.* **2.** *J. Antibiot. (Jap.)* **7A,** 15 (1954); *Jap. J. Med.* **6,** 493 (1953); *Chem. Pharm. Bull.* **6,** 539, 543, 548 (1958); *J. Antibiot. (Jap.)* **12A,** 133 (1959).
179	*NETROPSIN *S. netropsis* *S. chromogenus*	HCl: Long, thin, colourless hydrated prisms. Structure: β-[(4-guanidinoacetamidino-1-methyl-2-pyrrol-carboxamido)-1-methyl-2-pyrrolcarboxamido]-propionamide. $C_{15}H_{20}N_6O_3$	HCl: 167-72 dec.	238, 295. 236, 297	Opt. inact.	Pos. Sakaguchi, Ehrlich. Neg. ninhydrin, biuret, Tollens, Fehling, 2,4-DNPH, fuchsin-aldehyde, $FeCl_3$, amino-antipyrine.	Inhibits gram+, gram— bacteria, bacteriophages, vaccinia virus and Mecca lymphosarcoma in mice. Insectidal properties. *H. T.* **2;** *J. Am. Chem. Soc.* **79,** 1265, 1266 (1957); *Chem. Industr.* (1957) 365.

1	2	3	4	5	6	7	8
180	*AZOMYCIN *Nocardia* sp. resembling *N. mesenterica*	White needles. 2-Nitro-imidazole. $C_3H_3N_3O_2$	283 dec. 281-82 dec.	313 (EtOH) 314	Opt. inact.	Neg. $FeCl_3$, ninhydrin, biuret, Fehling, Molisch, Millon.	Inhibits gram+, gram— bacteria, Ehrlich carcinoma in mice. *H. T.* **2.**
181	*PUROMYCIN *S. alboniger* By synthesis	White cryst. Diacidic base. Structure: 6-Dimethylamino-9 [3'-p-(methoxy-L-phenylala-nylamino)-β-D-ribofuranosyl]-purine $C_{22}H_{29}N_7O_5$	175.5-177 uncor.	275 (0.1N NaOH) 267.5 (0.1N HCl)	—11(EtOH)	Neg. Brady.	Active against certain bacteria, protozoa, mouse mammary and brain tumours. *H. T.* **2**; *J. Am. Chem. Soc.* **80,** 2736 (1958).
182	*PSICOFURANINE (ANGUSTMYCIN C) *S. hygroscopicus* var. *angustmyceticus*; *S. hygroscopicus* var. *decoyicus*	Needles; white cryst. solid. 6-Amino-9-D-psicofuranosyl-purine. $C_{11}H_{15}H_5O_5$	202-04; 212-14 dec.	259 (0.01N acid) 261(0.01N base)	—71.1 (C_5H_5N) —53.7 (DMS) —68.0 (DMF)		*In vivo* activity against some bacteria and tumours. *H. T.* **2**; *J. Antibiot.* (*Jap.*) **11A,** 244 (1958) *Antibiot. and Chemother.* **9,** 432, 427, 436, 419, 675, 685 (1959).
182a	*TOYOKAMYCIN *S. toyocaensis*	Colourless needles. Weekly basic. Partially structure: Ribofuranoside of 4-amino-pyrrolo-[2, 3-d]-pyrimidine containing one nitrile group. $C_{12}H_{13}N_5O_4$	239-43 (monhydrate)	230, 279, 339. 230, 277 (H_2O). 255, 273 (HCl) 233, 280 (NaOH)		Neg. Fehling, biuret, $FeCl_3$. Pos. nynhydrin.	Active against yeast-like organisms, *Myco. tuberculosis*, and transplantable tumours in mice, *J. Antibiot.* (*Jap.*) **9A,** 60 (1956); *ibid.* **10A,** 189 (1957); *ibid.* **13A,** 361 (1960).
				ALICYCLIC CARBOXYLIC ACIDS			
	SARKOMYCIN A, A1, B	(*See* 58)					

1	2	3	4	5	6	7	8
		CYCLOHEXIMIDE — TYPE COMPOUNDS					
	CYCLOHEXIMIDE (ACTIDIONE) and isomers	(*See* 86, 87)					*J. Org. Chem.* 25, 344 (1960); *Chem. Pharm. Bull.* **8,** 335 (1960). *ibid.* 7, 654, 666 (1959).
	STREPTOVITACINS.	(*See* 88)					
183	*E-73 *S. albulus*	Colourless, narrow rectangular plates. 4-Acetoxycycloheximide. $C_{17}H_{25}NO_6$	140-41	285 (weak)	—8.8 (MeOH)		Inhibits sarcoma 180 in mice, HS-1 and HEp 3 in rats. *J. Am. Chem. Soc.* **82,** 1127, 1129 (1960).
	STREPTIMIDONE	(*See* 91)					Inhibits fungi, yeasts, protozoa, sarcoma 180. *J. Am. Chem. Soc.* **82,** 2974 (1960); *Antibiot. and Chemother.* **10,** 9 (1960).
184	*C-73 *S. albulus*	Pale yellow needles. Accompanies cycloheximide, E-73 B-73, fungicidin in broth. Carbon skeleton identical with that of cycloheximide and E-73. $C_{15}H_{17}NO_4$.	198-9	262, 365		Dark green colour with alc. $FeCl_3$. Bright yellow fluorescence under u.v.	Slight anti-tumour activity. *J. Org. Chem.* **25,** 661 (1960).
		QUINONOIDAL COMPOUNDS					
	CINERUBINS A, B	(*See* 113, 114)					

1	2	3	4	5	6	7	8
185	STREPTONIGRIN *S. flocculus*	Coffee brown to black long rectangular plates depending on solvent. pk_a 6.2-6.4 in 1:1 aq. dioxane soln. $C_{24}H_{22}N_4O_8$	275 dec.	c 247, 370 (MeOH)		Dark greenish brown with alc. $FeCl_3$; deep yellow with conc. H_2SO_4; dark greenish brown changing to red with evolution of NH_3 in aq. NaOH; in sodium hydrosulphite changes to yellow and reverts to brown on shaking with air; changes to yellow in Br-$CHCl_3$; orange red ppt. with 2, 4-DNPH.	Gram+, gram — and *Myco. tuberculosis* inhibited. High activity against several types of tumours in mice, rats and man *Antibiot. Ann.* (1959-60) 950; *Proc. Am. Assoc. Can. Res.* **3,** Abst. Nos. 97, 161, 194, 213 (1960).
				ALIPHATIC ACID AMIDES			
186	*LENAMYCIN *Streptomyces* sp.	Crystalline. Aliphatic compd. with an unsaturated bond, conjugated with carbonyl group, etheric C-O bond. $C_4N_4H_2O_{2-3}$	202-07 dec. 290-300 dec. unsharp on Al. block	216 (H_2O)		Neg. ninhydrin, biuret, anthrone, $FeCl_3$, Elson-Morgan, Sakaguchi, and tests for nitro and oxine.	HeLa (human) cervix carcinoma cell in tissue culture inhibited. *J. Biochem.* (*Jap.*) **45,** 9, 73, 159 (1958).
187	*CELLOCIDIN *S. chibaensis*	White cryst. Acecylenedicarboxamide. $C_4H_4N_2O_2$	216-18 dec., uncor.	299 (0.1N NaOH)		Identical with Aquamycin (C. A. **54,** 21314 d, 1960)	Active against *Mycotuberculosis* BCG, some gram+ bacteria, *E. coli* and mouse sarcoma. *J. Antibiot.* (*Jap.*) **11A,** 81, 84 (1958).
				POLYENES			
	ALIOMYCIN	(*See* 21)					
	MEDIOCIDIN	(*See* 39)					

1	2	3	4	5	6	7	8
			MISCELLANEOUS COMPOUNDS**				
188	MITOMYCIN *S. griseovinaceseus*	$C_{26}H_{33}NO_{12}$					Inhibits Yoshida sarcoma in mice. *C. A.* **51**, 9100h (1957)
189	MITOMYCIN A *S. caespitosus*	Reddish violet plates or needles. Basic. C 54.22, H 5.05, N 11.08, O 29.67	159-61 dec.	215, 316-18, 530 (H_2O) 360, 550-60 (0.1N NaOH) 235, 285, 335, 430 (0.1N HCl).		Reddish violet colour fades with Schiff reagent, 0.1N HCl and 0.1N NaOH, changes to blue with conc. H2SO4, and into orange in conc. HCl. Green colour with Molisch. Pos. 2,4-DNPH, Br. absorption; neg. Elson-Morgan, biuret, anthrone	Gram, + gram—, bacteria, Yoshida sarcoma and Ehrlich ascitic carcinoma inhibited. *J. Antibiot.* (*Jap.*) **9A**, 141, 147 (1956); *C. A.* **54**, 3869b (1069). *J. Antibiot.* (*Jap.*) **10A**, 120 (1957); *ibid.* **12A**, 146 (1959).
190	MITOMYCIN A-like compound *S. caespitosus*	Reddish violet substance. Labile in impure state. C 51.46, H 5.64, N 9.0, O 33.9.	167-68	216, 320, 520			Inhibits Ehrlich ascites tumour cells in mice. *Antibiot. and Chemother* **8**, 228 (1958).
191	FRACTION R *S. caespitosus*	Pink amorphous powder. No colour change with pH of soln.		215, 315			Inhibits cancer cells. *Antibiot. and Chemother.* **8**, 228 (1958).

**Mitomycin, Actinomycin, Ractinomycin, Chromomycin, Pluramycin seem to belong to the same group of pigmented antitumour antibiotics

1	2	3	4	5	6	7	8
192	FRACTION Y *S. caespitosus*	Orange cryst.	Browns 180; carbonizes 240	207, 237, 286, 345		Violet in alk. soln., yellow in acidic soln.	Antitumour activity. *Antibiot. and Chemother.* **8,** 228 (1958).
193	MITOMYCIN B *S. caespitosus*	Violet plates or needles. Basic.	182-84 dec.	220, 320, 550 (H_2O) 360, 580 (0.1N NaOH) 232, 284, 335, 430-40 (0.1N HCl).		Violet colour fades with Schiff and 0.1N HCl, changes to blue in 0.1N NaOH, blue to green in conc. H_2SO_4, yellow to orange in conc. HCl, green to brown in Molisch. Pos. 2,4-DNPH. Br. absorption; neg. Elson-Morgan, biuret, anthrone. C 53.11, H 4.94, N 8.92.	Activity similar to that of Mitomycin A(189). *J. Antibiot.* (*Jap.*) **9A,** 141, 147 (1956); *C. A.* **54,** 3869b (1960); *J. Antibiot.* (*Jap.*) **10A,** 120 (1957).
194	MITOMYCIN C *S. caespitosus*	Deep blue violet Cryst. $C_{54}H_{61}N_{13}O_{19}$	> 360	216, 360, 560		Pos. Fehling, hydroxylamine hydrochloride, biuret, Ehrlich, $FeCl_3$, nitrous acid, Liebermann, decolourizes $KMnO_4$, doubtful Br_2 absorption, 2,4-DNPH, Janovsky reactions. Neg. Benedict, Tollen fuchsin sulphate, ninhydrin, Millon, Raymond. Aq. soln. changes colour with pH. Bluish violet at alk., red at weakly acidic, yellow as acidity increases. Colour changes is reversilbe. Loses activity in sunlight irradiation.	Inhibits some bacteria, viruses and Ehrlich ascites mouse cancer. Clinical trials confirm antitumour activity. *Antibiot. and Chemother.* **8,** 228 (1958) *Antibiot. Ann.* (1958-59) 553; *J. Antibiot.* (*Jap.*) **12A,** 148 (1959).

1	2	3	4	5	6	7	8
195	LUTEOMYCIN (ANTIBIOTIC 289). *S. tanashiensis*, *S. flaveolus*	HCl: Orange yellow rhomboid. Basic. $C_{26}H_{33}NO_{12}$	199 dec. (HCl)	215, 255-57, 430		Colour changes from orange yellow to purple at pH 7-9. Ppld. as picrate, reineckate, helianthate and at low temp. as cryst. citrate. Pos. $FeCl_3$, quinone-Na_2CO_3, H_2O_2. Neg. xanthoproteic, biuret, Molisch, Fehling, ninhydrin, Sakaguchi.	Inhibits gram+, gram− bacteria, Yoshida sarcoma in rats. *H. T.* **2.** *J. Antibiot.* (*Jap.*) **12A**, 143 (1959).
196	PLURAMYCIN A *S. pluricolorescens*	Orange red needles (I) from EtOH; orange prisms (II) from ethylacetate-ether. (I) C 66.83, H 6.3, N 3.16; (II) C 66.87, H 6.61, N 3.8.	(I) darkens 177 (II) darkens 200-15	208, 245, 265-75		Purple in alk. soln., yellow in acid and aq. solns. Neg. $FeCl_3$, Fehling, Tollens, 2, 4-DNPH, nitroprusside.	Inhibits gram+ bacteria, and Ehrlich carcinoma in mice. *J. Antibiot.* (*Jap.*) **9A**, 22, 75 (1956); *ibid.* **10A**, 143, 213 (1957).
197	PLURAMYCIN N *S. pluricolorescens*	Reddish brown crude powder. Neutral.				Red colour with alkali	Inhibits *M. pyogenes*, and HeLa cells. *J. Antibiot.* (*Jap.*) **9A**, 22, 75 (1956).
	RACTINOMYCIN A	(*See* 108)					
198	GANCIDIN A *Streptomyces* sp.	Orange columnar cryst. Basic. $C_{43}H_{58-60}N_6O_{14}$	Darkens 290	206, 265, 340 (0.1N HCl)		Changes to pink at alk. reaction over pH 10. Pos. Fehling, Neg. ninhydrin, 2, 4-DNPH, $FeCl_3$. Some similarities to xanthomycin A.	Active against gram+ bacteria and Ehrlich cancer cells *in vitro* (contact test only). *J. Antibiot.* (*Jap.*) **9A**, 97 (1956); *ibid.* **11A**, 150 (1958).

1	2	3	4	5	6	7	8
199	GANCIDIN W *Streptomyces* sp. (produces Gancidin A)	White platelets. Neutral $C_{11}H_{17}$—$_{19}N_2O_2$	163-64	206 (0.1N HCl)		Neg. biuret, Ehrlich, Fehling, Benedict, Tollens, Molisch, 2, 4-DNPH, ferrous hydroxide, $FeCl_3$, Br addition.	Antitumour activity, *J. Antibiot.* (*Jap.*) **9A,** 97 (1955); *ibid.* **9B,** 160 (1956); *ibid.* **11A,** 150 (1958).
200	ABURAMYCIN *S. aburaviensis. Streptomyces* sp. M5-18903 gives the optical antipode	Yellow cryst. Weakly acidic. C 56.32, 55.57; H 7.44, 7.54;	163-65 169-71 (M5-18903)	230, 276, 410	+24.56 (MeOH) —29 (MeOH) M5-18903	Pos. Molisch, Fehling, Benedict, Tollens, Seliwanoff, anthrone. Neg. biuret, xanthoproteic, ninhydrin, Tollens, $FeCl_3$. Folin, tyrosine.	Inhibits some gram+ bacteria, *E. histolytica* and *Syphacia obvelata* in mice. Some activity against Ehrlich ascities, Crocker sarcoma and leukemia in mice. *J. Antibiot.* (*Jap.*) **10A,** 205, (1957); *Antibiot. Ann.* (1958-59) 489; *Hindustan Antibiot. Bull.* **2,** 120 (1960).
201	ACTINOBOLIN *Streptomyces* sp.	Amorphous, white, fluffy powder. Hygroscopic. Amphoteric with a basic function of pK_a 7.5 and a weakly acidic (enolic) function of pK_a 8.8. $C_{13}H_{20}$—$_{22}N_2O_6$.		263 (phosphate buffer)	+54.5 (sulphate in H_2O).		Inhibits gram+, gram— bacteria, and several types of tumours *in vivo*. *Antibiot. Ann.* (1958-59) 497, 505, 510, 515, 522, 528.
202	SULFOCIDIN *Streptomyces* sp.	White, amorphous (crude); yellow brown cryst. Neutral. Probably contains an α, β-unsaturated ketone system. C 62.0, H 7.50, N 3.1, S3.6.	166-78.	End absorption with shoulder at 282 (EtOH)			Gram+, gram—, acid fast bacteria inhibited. Moderate activity on fungi. Inhibits ascites forms of Gardner lymphosarcoma 180 in mice. *Antibiot. Ann.* (1957-58) 886, 972.

1	2	3	4	5	6	7	8
203	MUTOMYCIN *S. atroolivaceus var. mutomycini*	White needle shaped cryst. powder. Mol. wt. 124. C_7H_{11}—$_{12}O_2$	141.5-142				Inhibits *Staphylococci*. Slight activity on Ehrlich carcinoma in mice. *Antibiotics* (*Trans.*) **4**, 273 (1959).
204	MARINAMYCIN *S. mariensis*.	Pale yellow hygroscopic amorphous powder. Not absorbed on active charcoal, Al_2O_3, silica gel, starch, diatomaceous earth, ion exchangers.				Pos. ninhydrin, Sakaguchi, Folin. Neg. indole, diazo, Bial; ambiguous positive biuret, Fehling.	Active against Ehrlich ascites and solid tumours. Low activity against some bacteria. *J. Antibiot.* (*Jap.*) **12B**, 300 (1959).
	HYGROSCOPIN B.	(*See* 89)					
205	CARYOMYCIN *S. filamentosus*						Inhibits Yoshida sarcoma in rats. *J. Antibiot.* (*Jap.*) **6A**, 156 (1955).
206	RAROMYCIN *Streptomyces* sp.						Ehrlich carcinoma, and Crocker sarcoma 180 in mice inhibited. *J. Antibiot.* (*Jap.*) **10A**, 189 (1957).
207	ACTINOXANTHINE *Actinomyces globisporus*						Inhibits Ehrlich carcinoma and *M. pyogenes* var *aureus*. *Antibiotiki* **3**, No. 1, 18, 22, 27, 31, 36, 40 (1958).
208	PA-144 *Streptomyces* sp.						Human and animal embryonic cell carcinoma responded. *Proc. Am. Assoc. Can. Res.* **3**, *Abst.* 47 (1960).

Index to Melting/Decomposition Points (Range in °C)

M. P.	100-05	105-10	110-15	115-20	120-25	125-30	130-35	135-40	140-45	145-50
S. NO.			86 87	86		88			183 203	
M. P.	150-55	155-60	160-65	165-70	170-75	175-80	180-85	185-90	190-95	195-200
S. No.	153* 154*	88 113	189 199 200	179* 190 200	202*	181 196*	114* 193	173	160	184 195*
M. P.	200-05	205-10	210-15	215-20	220-25	225-30	230-35	235-40	240-45	245-50
S. No.	182 186	108*	160 182 196*	172 187	170* 178*		161	161	182a 163 114* 192* 161	113* 164
M. P.	250-55	255-60	260-65	265-70	270-75	275-80	280-85	285-90	290-95	295-300
S. No.	162 165 167	166			91	185	157 180		186 198*	
M. P.	300-05	305-10	310-15	315-20	320-25	325-30	330-35	335-40	340-45	345-50
S. No.										
M. P.	350-55	355-60	360-65	365-70	370-75	375-80	380-85	385-90	390-95	395-400
S. No.			156							

Remarks on aesterick (*) marked numbers:

(108) Blackens.
(113) Solidifies in rosette needles which disappear at 249°.
(114) Solidifies about 168-78 and disappears at 240-43°. 180° in capillary.
(153) 142-62 dec.
(154) 145-55 dec.
(170) K salt.
(178) Chars about 220°.
(179) Hydrochloride.
(186) 290-300 unsharp on Al block.
(192) Carbonizes at 240°.
(195) Hydrochloride.
(196) Darkens.
(198) Darkens.

INDEX TO U. V. ABSORPTION MAXIMA

λmax. (mμ)	0	1	2	3	4	5	6	7	8	9
210	170 198 192 199 196					189 191 195	186 190 194	171	172	
220	193									
230	58 182a 200		91 193	89 182a		113 114 139	166 179	192	179	
240	163 165	163	156	160	154 164	108 196		185		
250	153 173		153			182a	195		114	113 182
260		182	184	201		168[1] 198			181	
270	196[2]			182a	154 155	181	200	78		182a
280	182a 170			172	193	163 183 189	192			
290	91 171				113 114	179		179		187
300							39			
310			160		180	191		189		
320	21 190 193	39								
330	21					189 193				182a
340	39 198				163	192				
350		21					39			
360	189 193 194	49				161				
370	185							39		
380		49								
390										
400					49					
410	200									
420			163							
430	189					193[3]				

λ max (mμ)	0	1	2	3	4	5	6	7	8	9
440	165					108[4] 163 164			166	
450	167									163
460										
470				113 114				163		
480								113	114	
490								113 114		
500										
510									113	114
520	190									
530	189		114	113						
540										
550	193					189[5]				
560	194									
570										
580	193									

(1) 260-70 mμ. (2) 265-75 mμ. (3) 430-40 mμ. (4) 440-50 mμ. (5) 550-60 mμ.

FORMULA INDEX

Formula	*Melting Point* (°C)	*S. No. of Antibiotic*
$C_3H_3N_3O_3$	281-82 dec., 283 dec.	180
$C_4H_4N_2O_2$	216-18 dec., uncor.	187
$C_4H_4N_2O_2$—3	202-07 dec., 290-300 dec., unsharp on aluminium block	186
$C_5H_7N_3O_4$	142-62 dec., before melting	153
$C_6H_9N_3O_3$	144-55 dec., with gas evolution	154
$C_7H_8O_3$	..	58
C_7H_{11}—12O_2	141.4—142	203
$C_{11}H_{15}N_5O_5$	202-04; 212-14 dec.	182
$C_{11}H_{17}$—19N_2O_2	163-64	199
$C_{12}H_{13}N_5O_4$	239-43 (monohydrate)	182a
$C_{13}H_{20}$—22N_2O_6	..	201
$C_{15}H_{20}N_6O_3$	167-72 dec. (hydrochloride); 224-25 (sulphate)	179
$C_{15}H_{17}NO_4$	..	184
$C_{15}H_{21}N_7O_6$	..	155
$C_{15}H_{23}NO_4$	109-11, 112, 115-116.5, 210	86
$C_{15}H_{23}NO_4$	109-110	87
$C_{15}H_{23}NO_5$	156-61, 124-28 (isomer); 100-02 (isomer)	88
$C_{15}H_{28}N_2O_3$	B $P_{0.009}$ 70	89
$C_{16}H_{23}NO_4$	272-73	91
$C_{17}H_{16}N_2O_6$	Darkens c. 180, chars c. 220	178
$C_{17}H_{25}NO_6$	140-41	183
$C_{22}H_{29}N_7O_5$	175.5-177 uncor.	181
$C_{24}H_{22}N_4O_8$	275 dec.	185
$C_{26}H_{33}NO_{12}$	..	188
$C_{26}H_{33}NO_{12}$	199 dec. (hydrochloride)	195
$C_{29-30}H_{40-42}N_6O_{7-8}S$	191-92 dec., 213 dec.	160
$C_{33}H_{30}H_3O_{14}$	Browns at 157-58, blackens at 205	108
$C_{33}H_{60}$—62NO_{14}	189-90	173
$C_{43}H_{58}$—60N_6O_{14}	Dark orange at 110 and black at 290	198
C_{43}—45H_{57}—60NO_{18}	155-58 solidifying at 160-88 in rosette needles which disappear above 249	113
C_{43}—45H_{57}—61NO_{18}	168-78 solidifying on further heating and disappears at 240-43. 180 (capillary)	114
$C_{54}H_{61}N_{13}O_{19}$	Does not melt by 360	194
$C_{60}H_{76}H_{12}O_{15}$	241.5; 243 dec.	163
$C_{60}H_{83}N_{11}O_{16}$	..	161
$C_{62}H_{89}N_{11}O_{17}$	..	161
$C_{64}H_{90}N_{12}O_{16}$	232-35 dec.	161

SHORT NOTES

Hamycin in the Treatment of Otomycosis

(Preliminary observations)

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OTOMYCOSIS is a chronic or subacute infection of the external auditory meatus and ear canal, characterized by exudative inflamation and pruritus, and from which can be isolated a number of fungi and bacteria. Fifty-three different species have been reported as etiologic agents of this condition. Among these, *Aspergillus niger* is the commonest pathogen frequently met with in our department.

Hamycin‡ has very high activity (1 in 100 million parts), against *A. niger*. It was, therefore, used in the treatment of otomycosis caused by *A. niger* to evaluate its clinical use in this condition. The first group of twenty-five cases of otomycosis in which this antibiotic was used is reported in this paper.

MATERIALS AND METHOD

Material

Twenty-five cases of fungal infections of the ear canal were selected from out-patients-department of E.N.T., Sassoon Hospitals, Poona. Twenty-four patients were males and 1 was a female. Twenty-one patients had unilaterial infection of the ear canal, and 4 had bilaterial infections (Table I).

Method

In each case a swab from the affected ear canal was taken under aseptic precautions for microscopic examination. Epithelial debris and the material collected from the ear canal was cultured on Sabouraud glucose agar. The positive cases were treated with Hamycin glycerine suspension of 0.1% concentration. Hamycin was applied locally with a sterile swab two times a day.

Clinical symptoms

All the cases complained of intense itching and pain of the ear canal. Inflammation, erythema, a sense of fullness due to mechanical obstruction and moderate discharge in 3 cases were the other associated symptoms observed.

Mycological examinations

The swab from the affected ear canal was taken and the material mounted on a slide in 10 per cent KOH solution. Microscopic examination of the preparation under a cover glass revealed broken septate hyphae, spherical one celled conidia varying from brown to blackish in colour, and characteristic vesiculose condiophores of *Aspergillus* sp.

Culture

The material was then cultured on Sabouraud's glucose agar and cultures were incubated for a week at 28°. In 18 out of 23 cases *A. niger* was found to

* Department of Ear, Nose and Throat. ** Department of Dermatology, Mycology Unit.

‡ Trade name of Hindustan Antibiotics Ltd., Pimpri for an antifungal antibiotic from *Streptomyces pimprina*.

TABLE I

Infection of auditory canal		Infection of the ear canal and also external auditory meatus		Total
Unilateral	Bilateral	Unilateral	Bilateral	
19	2	2	2	25

be the causative organism and in 2 cases *A. glaucus* was noted.

Results of treatment

All the 25 cases were treated with Hamycin glycerine suspension (0.1 per cent). The drug was applied locally with a sterile cotton swab two times a day.

In 16 cases the intense itching and pain subsided after 4-7 days of treatment and in the remaining 9 cases after 8 to 10 days. The clinical cure in 16 cases was observed after 7-9 days and in 9 cases after 10-14 days. All the cases were followed after every 4 days. The follow-up cultures in all cases were positive on 5th day. In 21 cases the cultures were negative by 8th day, and in all cases the cultures became negative by 14th day. All the cases were followed for 2 months after the completion of treatment. No relapse was observed. No untoward effects were noted in this series.

DISCUSSION

No attempt was made to compare the efficiency of the drug with other drugs commonly used in the treatment of this condition. However, with other common treatments such as thymol solutions or 2 per cent salicylic acid it is observed that relapses are common. Because of the fungicidal property of the Hamycin, majority of the cases showed no relapse in this series. Hamycin was found to be very effective, and also easy to apply. No untoward side effects were noted. A detailed control study on large scale is under progress.

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We wish to thank Dr. B. B. Gokhale, Honorary Dermatologist and Venereologist, for making available the experimental quantity of Hamycin. Our thanks are also due to the authorities of Hindustan Antibiotics Ltd., Pimpri and Sassoon Hospitals, Poona, for their help and co-operation.

Colorimetric Method for Determination of Penicillin

I. DETERMINATION OF PENICILLIN IN PROCAINE PENICILLIN G.

G. R. DESHPANDE & S. S. KARMARKAR
Research Laboratories, Hindustan Antibiotics Ltd., Pimpri, Near Poona

SEVERAL methods are known for the estimation of penicillin, the bioassay[1], iodometric method[2], and hydroxylamine hydrochloride (colorimetric)[3] method being the most widely used. The first two methods are sufficiently accurate but time consuming in practice. The third method is not very sensitive. We have developed a quick and sensitive colorimetric method using phosphomolybdic acid reagent for estimation of penicillin. The blue-green colour[4] which develops is quite stable for several days (Fig. 1) facilitating overnight estimations. Further, the colour has only one absorption peak at 720 mμ (Fig. 2) in the visible region permitting estimations even with filter type colorimeters.

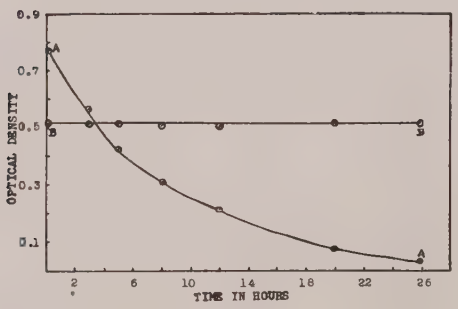

Fig. 1. A: Stability in the presence of $NaHCO_3$. B: Colour stability curve.

In the present paper, estimation of penicillin in procaine penicillin G by the above method is described.

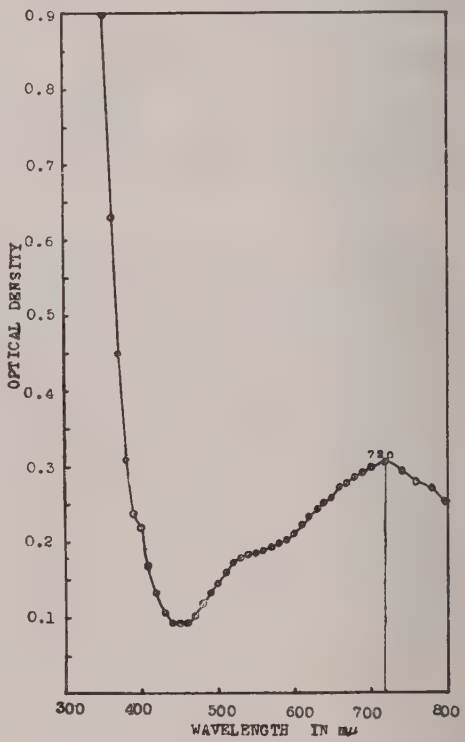

Fig. 2. Absorption spectrum.

MATERIAL AND METHODS

Beckman model DU spectrophotometer with 1 cm. cells. ¶10% phosphomolybdic acid aqueous reagent (E. Merck, pro-analysis quality, stocked in amber coloured bottle ; stable indefinitely).

Standard potassium penicillin (aqueous), about 400 u/ml. (Dissolve 16 mg. in 50 ml. water. Prepare fresh each time).

Plotting the calibration curve

With a pipette add about ten aliquots of standard penicillin solution into 10 ml. graduated test tubes using volumes ranging from 0.1 ml. to 1.0 ml. To each test tube add phosphomolybidic acid reagent (0.4 ml.) and then water to make the volume to 1.4 ml. in each tube. Keep the tubes in boiling water bath for exactly 10 min., then cool to room temperature and dilute with water to 10 ml. mark. The absorption at 720 mμ is read off on the spectrophotometer. Correct the absorbance reading for reagent blank and plot the absorbance against penicillin concentration (Fig. 3).

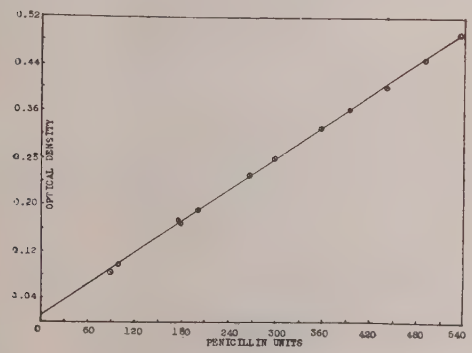

Fig. 3. Concentration curve (Wavelength 720 mμ).

Procedure for determination of penicillin in procaine penicillin G.

Weigh about 20 mg. of procaine penicillin accurately and dissolve in distilled methanol (5 ml.), and dilute with water to 50 ml. in a volumetric flask. With a pipette add 0.5 ml. solution, in duplicate, into 10 ml. graduated test tubes. Add reagent (0.4 ml.) and water (0.5 ml.) Keep in boiling water bath for 10 min. (The time factor was calculated from the colour development curve, Fig. 4). Cool to room temperature and dilute with water to 10 ml. mark. A yellow precipitate forms in the test tubes simultaneously with the development of the blue-green colour. The nature of the precipitate is under investigation. Centrifuge the solution for 2 min. at 1,500 r.p.m. Read off the absorbance on the spectrophotometer, and calculate the penicillin unitage from the calibration curve. (Fig. 3).

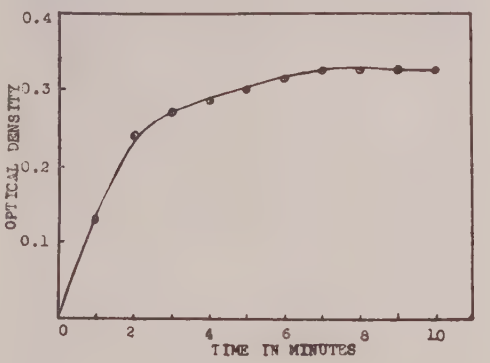

Fig. 4. Time curve for colour development.

A number of procaine penicillin samples have been analysed by the new method and the results compare favourably with those obtained by the iodometric method (Table I).

TABLE I. PENICILLIN IN PROCAINE PENICILLIN G DETERMINED BY NEW COLORIMETRIC METHOD AND BY IODOMETRY

Batch No.	Penicillin in units		Difference between (2) and (3)
	By iodometry	By new colorimetric method	
(1)	(2)	(3)	(4)
1	997.0	1,007.0	+ 10
2	998.0	1,000.0	+ 2
3	999.0	1,002.0	+ 3
4	997.0	997.7	+ 0.7
5	999.0	1,000.0	+ 1
6	999.0	998.0	— 1
7	999.0	1,010.0	+ 11
8	998.0	1,006.0	+ 8
9	999.0	1,001.4	+ 2.4
10	998.0	1,005.0	+ 7
11	1,001.0	1,001.0	0
12	1,001.0	1,004.0	+ 3
13	997.0	994.0	— 3
14	999.0	998.2	— 0.8
15	997.0	989.0	— 8
16	998.0	997.0	— 1
17	999.0	997.7	— 1.3

Application of the method for the determination of penicillin in other penicillin salts and process samples will be reported elsewhere.

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I, Shantikumar T. Raja, hereby declare that the particulars given above are true to the best of my knowledge and belief.

Dated : 15th April, 1961

(Sd.) S. T. Raja,
for Hindustan Antibiotics Ltd.,
Pimpri, Poona Dist.

PORTRAIT OF A COMPANY

6. Eli Lilly and Company, Indianapolis, Indiana, U.S.A.

DAVID RICHARDS

THE discovery of penicillin by Sir Alexander Fleming in England in 1928 and its development into a mass-produced drug in the United States in the 1940's opened up a new field in the treatment of many infectious diseases for which there had previously existed no known cure. This field of therapy has been developed and enlarged immensely by cumulative efforts of scientists employed by pharmaceutical houses, universities, and the United States Government; today there are over 100 different antibiotic preparations, of which penicillin is still the most important one in use. As a result of these great advances, some of the world's most common causes of death and disability have been brought under control.

Eli Lilly and Company played an active part in the development and production of penicillin from the very beginning. Early in 1942, the United States Government asked a number of pharmaceutical companies to make every effort to manufacture the drug ; full-scale production started at Lilly in 1943. After the end of World War II, the demand for antibiotics grew sharply, and the company expanded its facilities accordingly. In the 1950's, a $20 million plant with a fermenting capacity of 432,000 gallons was erected at Lafayette, Indiana. At the present time, Eli Lilly and Company is marketing seven basic types of antibiotics — penicillin, streptomycin, erythromycin, vancomycin, cycloserine, neomycin sulfate, and bacitracin. More than 90 antibiotic items are included in the company's price list.

The discovery of new antibiotics is time-consuming, but it is also urgent business. The wheels of medical progress quickly roll over any drug company that dared to rest on its research and sales labors of the past without striving to improve the products already in use. Through its research efforts, Eli Lilly and Company has made four major contributions to this field.

The early form of penicillin was crude by today's standards. The drug had to be administered by the drip method ; and since it contained many impurities, it was painful to administer. Even after injection of penicillin had become common, hospitalization of the patient was often necessary. After many attempts to find a penicillin requiring only one daily injection, Lilly scientists produced procaine penicillin G. Marketed in 1948 under the trademark Duracillin* the drug became the standard one-dose-per-day injectable penicillin, and remains so today.

The second major contribution came in 1952 when the company marketed its discovery erythromycin under the trademark Ilotycin.* More than 100,000 microorganisms were isolated from soil samples and tested before the one producing Ilotycin was found ; its source was a sample of earth collected in the Philippine Islands some five years earlier.

This new medicine was particularly effective against staphylococcal infections. In addition, unlike some other antibiotics, it had no adverse effect on the bacteria which normally inhabit the intestine and which are necessary for the normal functioning of the body. A new form of the drug, which produces greater antibacterial activity in the blood stream, was marketed under the trademark Ilosone* in 1958.

During the early 1950's, penicillin therapy continued to advance, and scientists were searching for an oral form of the drug. In 1955, Lilly introduced its V-Cillin* products which are now widely used in oral penicillin therapy.

* Registered trade mark.

The company's latest contribution came in 1958 when it marketed one of the most powerful bactericidal antibiotics in history under the trademark Vancocin.* Work on this product started in Indianapolis in 1952 when a soil sample, collected by a Christian missionary in a village of Borneo, arrived in the company's research laboratories. The drug has been literally a lifesaver for patients critically ill with infections resistant to other antibiotics. Many remarkable recoveries have taken place.

This is a fast-moving century. Yet, the conquest of disease—one of the basic goals of Eli Lilly and Company — is still one of the most insistent needs of mankind. Despite the great progress of medical science in this century, surpassing the advances of the previous 2,000 years, man is still plagued by heart disease, cancer, virus infections, mental ills, parasitic infections, metabolic defects, and malnutrition.

Some of these health problems have enormous dimensions. About 800 million persons, close to one-third of mankind, suffer from worm parasites. Conscious of the need in this area, Lilly research resulted in the production of an anthelminthic known as Telmid.* There are an estimated 10 million lepers in the world today. Malaria is still a scourge in many nations, sapping the vigor of their populations, although the World Health Organization has declared war against the disease and hopes to eradicate it by 1968. Epidemics of cholera, banished from all other parts of the world, still occur in India, Pakistan, Nepal, and Thailand.

In the Lilly Research Laboratories in Indianapolis, the scientific staff is now working in some sixty project areas covering virtually every field of human medicine. Cancers, cardiovascular diseases, and mental illnesses are among the major areas of interest. A large cancer research program screens some 5,000 to 7,000 compounds each year for antitumor activity ; several promising agents are under study, including an alkaloid from a garden shrub which has shown good activity in the palliative treatment of at least one type of cancer. Altogether, more than 1,300 men and women are engaged in the research, development, and control activities of the company.

The search for new antibiotics is also intensive. In a recent ten-year period, Lilly scientists screened 10,000 soil samples (many of them gathered from the farthest corners of the world) and isolated 200,000 organisms. Of these organisms, 25,000 showed some antibiotic activity. Seven eventually reached clinical trial ; and of these, three produced new products.

The discovery of new medicines is an expensive business and becomes increasingly so as the years go by. Every drug company with a major research program is faced with this economic fact of life. In 1950, Lilly's research and development budget was no more than $ 4.1 million ; during 1960 the company spent $20 million on its research activities — a growth of nearly 500 per cent. These expenditures have borne fruit. During the decade of the 1950's, Lilly's broad scientific research programe resulted in the introduction of seventy new products, each representing a significant advance in the treatment of disease.

Eli Lilly and Company, one of oldest American manufacturers of prescription medicines, had its origin eighty-four years ago in a small room in Indianapolis, Indiana, where its founder, Colonel Eli Lilly, produced medicines for use by the physician. Colonel Lilly's capital was some $1,300 ; and his staff was composed of three employees, including his 14-year-old son, J. K. Lilly, who later becam president of the company and served in that position for thirty-four years.

The company has grown and prospered to become one of the world's leading pharmaceutical houses. A major turning

* Registered trade mark.

point in this growth came in 1923 when Lilly developed the first large-scale production of Insulin, which had been discovered earlier by Dr. Frederick G. Banting and Dr. Charles H. Best in Canada. Today there are eight million known diabetics in the world for whom active and useful lives are possible with the use of this product.

Lilly's four manufacturing plants and agricultural research farm cover more than 1,000 acres in the State of Indiana, while veterinary medicines are manufactured in Omaha, Nebraska, by one of the company's subsidiaries, Corn States Laboratories, Inc. In the international sphere, Lilly is presently engaged in an expansion program. Apart from its existing facilities in Argentina, Australia, Brazil, Canada, Colombia, England, Mexico, and India (with a sales office in Bombay), Lilly has recently established subsidiaries in Italy, Switzerland, and West Germany and is in the process of forming companies in France and Venezuela. The company employs about 10,000 persons of which 2,000 are located in fifty nations outside the United States. Sales volume abroad is moving upward, and Lilly products can now be bought in 126 countries in the free world.

Not only are Lilly's production and sales facilities organized on a world-wide basis, but the company's international scope is also extending into the areas of research and clinical trials.

In the fall of 1959, Lilly signed an agreement with the Syntex Corporation of Mexico City which enables the company to participate in the important and growing steroid market. Under the agreement, Lilly will conduct clinical investigations of promising new compounds discovered in the Syntex Laboratories.

A cancer drug is at present undergoing clinical trials both in the United States and in several other countries. Clinical trials on an international scale are not uncommon these days, and Lilly is testing a number of other products in this manner.

Although Eli Lilly and Company is best known as an ethical drug manufacturer, it has taken a number of major steps in the agricultural field during the past few years. The first came in 1954 with the organization of the Agricultural and Industrial Products Division. In June, 1959, the company dedicated a $5 million Agriculture and Veterinary Medicine Research Center in Greenfield, Indiana, where all the modern facilities necessary to carry on research in animal and plant nutrition, disease, and pest control are available.

Guided by a staff of 120 persons, research projects are in full swing at the Greenfield center. Feed additives are being tested on swine, cattle, sheep, and poultry. Some of these additives stimulate weight gains ; others increase feeding efficiency. Veterinarians are working on the prevention of various animal diseases. Other scientists are looking for chemicals that will stimulate or retard plant growth, and concentrated effort is being made to discover new insecticides and pesticides. The goal of the research center is to help solve the problems which are costly to farmers in crop and animal losses.

Early in 1960, the company formed Elanco Products Company for the purpose of marketing items in the fields of agriculture, industry, and home use. About the same time, Lilly entered the plastics business by acquiring Diamond Plastics Industries, Inc., one of the major producers of plastic boxes in the United States.

Despite this diversification into various new fields, the fundamental objective of Eli Lilly and Company remains the discovery and production of high quality prescription medicines for human use. This has been the basic aim of the company since it was founded. As Eugene N. Beesley, president of the company, recently said, "We have our eyes fixed on a challenging goal — to do everything we can, by creating supplies of safe and effective medicines, to ease human suffering and eliminate the diseases that plague mankind."

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